TRANSFORMATIONS OF ETHYLENE DIBROMIDE IN AQUEOUS SYSTEMS AND GROUNDWATER

By

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KEY TO SYMBOLS AND ABBREVIATIONS

ACS	American Chemical Society
aq	aqueous phase
BDS	butyldisulfide
°C	degrees Celsius
^{14}C-	carbon 14 isotope labeled chemical
ca.	approximately
CHT	cyclohexylthiol
cm	centimeter
cpm	counts per minunte
d	day
dpm	disintegrations per minute
DT	1,4-dithiane
DTT	1,3-dithiolane-2-thione
EDB	ethylene dibromide
EPA	Environmental Protection Agency
ET	1,2-ethanedithiol
eV	electron volts
g	gram
hr	hour
H_2S	hydrogen sulfide
i.d.	inside diameter
°K	degrees Kelvin

KEY TO SYMBOLS AND ABBREVIATIONS
(Continued)

L	liter
M	mole per liter
m	meter
mCi	microcurie
m/e	mass-to-charge ratio
min	minute
mL	milliliter
mol	mole
ND	none detected
pg	picogram
T	temperature
TT	1.3.5-trithiane
TTCD	1,4,7,10-tertathiacyclododecane
TTCH	1,2,5-trithiacycloheptane
TTCO	1,2,5,6-tetrathiacyclooctane
TTCP	1,2,3-trithiacycolpentane
U-	uniformly radiolabeled chemical
μg	microgram
μM	micromole
yr	year

Abstract of Dissertation Presented to the Graduate School at the University of Florida in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

TRANSFORMATIONS OF ETHYLENE DIBROMIDE IN AQUEOUS SYSTEMS AND GROUND WATER

By

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Registration of a chemical for use as a pesticide today requires thorough study of the compound's environmental fate in water, soil, and air to determine if classification restricting its use is needed. During the 30-year history of "successful" soil fumigation with ethylene dibromide (EDB, 1,2-dibromoethane), its environmental fate was not closely studied. This nematicide was not recognized as environmentally persistent or a public health risk until 1983, when its agricultural use was suspended in the United States.

In this research, the transformations of EDB were examined both in buffered aqueous solutions and in groundwater from several locations in Florida. Experiments were conducted between 25 and 70°C to establish temperature-dependent rate constants and to expedite the reactions. The ethylene dibromide and products were analyzed by packed

and capillary gas chromatography (GC), liquid scintillation counting, and a colorimetric assay. The EDB is converted to ethylene glycol and bromide ion in aqueous solutions and in abiotic aerobic groundwaters. The rate of degradation of EDB is dependent upon temperature but independent of pH between 5 and 9. Extrapolating experimental results to the average ground- water temperature in Florida of 22°C, the degra- dation half-life of EDB in the tested groundwaters is 1.5 to 2 years.

An alternative transformation of EDB occurs under anaerobic reducing conditions in groundwater where aqueous hydrogen sulfide ($H_2S_{(aq)}$) is commonly present. Transformation by $H_2S_{(aq)}$ was not previously recognized as a potentially important reaction with pesticides. The rate of degradation of EDB under these conditions is increased and dependent upon temperature, pH, and $H_2S_{(aq)}$ concentration. The sulfur-containing products are 1,2-ethanedithiol and saturated cyclic rings of one, two or four ethylene units and two, three, or four sulfur atoms. Their structural identity was elucidated with mass spectrometry (MS) and GC retention times of products relative to known analytical standards. One commercially unavailable cyclic disulfide compound was synthesized and used to positively confirm the MS and GC findings. Toxicity assessment of sulfur-products, by Microtox™ screening assay, suggests increased toxicity relative to parent compound or to its hydrolysis products.

INTRODUCTION

The reported episodes of groundwater contamination by landfills and septic tanks, hazardous waste sites, petroleum-based hydrocarbons, halogenated hydrocarbons, and pesticides have dramatically increased in the United States in recent years (Pye and Patrick, 1983). These reports were followed by efforts to address the problem at both the federal and state levels. An official report from the U.S. Environmental Protection Agency (EPA) in 1984 stated that "significant achievements have been made nationally in the protection and enhancement of water quality" by controlling the many point sources of pollution (U.S. EPA, 1984a, p. 2). This report also recognized the contributions of nonpoint sources to contamination of groundwater. The later term refers to pollutants which originate principally from agricultural activities, runoff from urban lands, and mining activities. In an Association of State and Interstate Water Pollution Control Administrator's Nonpoint Source Pollution Survey, 78% of the states indicated that the magnitude of nonpoint source pollution problems was at least as great as that of point source problems (U.S. EPA, 1984b). In all regions studied by the EPA, agricultural activities in particular were identified as the most widespread nonpoint

source of groundwater contamination, affecting 50% of the waters in 72% of states (U.S. EPA, 1984b). The severity of the problem depends to a large extent upon local factors including the hydrogeology of the region, agricultural practices, rainfall, terrain, and the particular chemicals that are dispersed into the environment.

Of all the agricultural pollutants of water, pesticides are the compounds posing the greatest toxicologic hazard. Of particular interest recently, and the focus of this research, is the nematicide fumigant, 1,2-dibromoethane or ethylene dibromide (EDB). It had been widely used in many states. By 1984, the chemical had been detected in the groundwater of at least six states. While EDB was first registered for agricultural use by the U.S. Department of Agriculture in 1948, only relatively recently has extensive information been available on the toxicological properties of this chemical.

The literature review which follows presents current knowledge of the persistence, fate, and transport of EDB in the environment and the evolution of the discovery of this problem. The state of Florida has had particular interest in this episode for several reasons that will be discussed. Close study of the history of EDB usage underscores the urgency of evaluating and amending current practices that may lead to groundwater contamination, especially from unexpected nonpoint sources such as pesticides. Prior to

discoveries of EDB in groundwater, the chemical was thought to be sufficiently volatile and of sufficiently low water solubility to not present an environmental hazard. This belief is reflected in a report prepared for EPA in 1975 which states "ethylene dibromide is not believed to accumulate in the environment. Limited information suggests that it degrades at moderate rates in both water and soil" (Going and Long, 1975, p 1b). Review of the literature suggests that only until relatively recently was the movement and accumulation of EDB in the subsurface of interest. Beckman et al. attributed increased bromide content in various crops to soil pre-treatment with nematocidal compounds, EDB and 1,2-dibromo-3-chloropropane, which were "readily degradable," liberating inorganic bromide into the soil (Beckman et al., 1967, p 138).

The detection of EDB in groundwater came about through increased sensitivity of modern analytical chemistry instrumentation and a renewal of concern for environmental contamination by man's daily practices. In the 1960s, residual EDB was determined by measuring inorganic bromide in soil and plant tissue (Beckman et al., 1967; Mapes and Shrader, 1957). These indirect methods involved combustion or oxidation of EDB followed by assay of bromide by titration or polarography. Determination of greater than 1 to 10 parts per million (ppm) was possible; however, these values were highly dependent upon the care taken to

establish the background halide content. In the 1980s, EDB is detected directly in agricultural commodities (Rains and Holder, 1981; Morris and Rippon, 1982) and environmental matrices (Amin and Narang, 1985; Marti et al., 1984) at concentrations below 10 parts per billion (ppb). These methods employ purge and trap gas chromatography and mass spectrometry for achieving lower detection limits and identity confirmation.

With the benefit of modern analytical chemistry instrumentation, this study was designed to achieve the following goals: (1) to describe the kinetics of degradation of EDB in buffered aqueous systems and in groundwater, (2) to elucidate the major products of EDB degradation in groundwater, and (3) to investigate EDB degradation occurring in buffered aqueous systems and groundwater in the presence of aqueous hydrogen sulfide ($H_2S_{(aq)}$), a reactive, natural component in groundwater.

LITERATURE REVIEW

Groundwater Contamination by Pesticides

The Environmental Protection Agency recognized the need for investigation of groundwater contamination by pesticides in 1979. The first pesticides discovered in groundwater of various states were 1,2-dibromo-3-chloropropane (DBCP) and aldicarb, the later occurring mostly as the sulfone or sulfoxide (Cohen et al., 1984). Over 50 different pesticides were found in ground-water basins of California, one of the largest users of pesticides in the nation. One of the best known incidences was the discovery of DBCP in hundreds of wells in the San Joaquin Valley (Litwin et al., 1983). In 24 other states, a total of 19 pesticides were detected in groundwater (U.S. EPA, 1987b).

The importance of the safety of groundwater is evident when faced with the fact that it provides at least half the potable water to the U.S. population (Pye and Patrick, 1983). The gravity of the situation may be even more acute in locations such as Florida, where groundwater provides 92% of the population's potable water supply (Fernald and Patton, 1984; U.S. EPA, 1987a).

The state of California, in collaboration with the U.S. EPA in the early 1980s, studied the extent and mechanism of

groundwater contamination by four widely used pesticides: DBCP, EDB, simazine, and carbofuran. EDB was found at concentrations between 0.1 and 31.1 ppb in soils at depths greater than three meters. Numerous wells were also found to be contaminated (Zalkin et al., 1983). Meanwhile, in the summer of 1982, irrigation wells in Georgia were found to contain EDB at levels as high as 100 ppb (Marti et al., 1984). The greatest extent of contamination, however, was not uncovered until the state of Florida began monitoring groundwater wells in the summer of 1983. In Florida, contamination was found to exist throughout much of the heavily farmed regions of the state. Concentrations in private wells as high as 900 ppb were reported[1]. Florida responded rapidly by suspending the use of this agrochemical in July, 1983 (Florida Statutes, 1983). The EPA followed this action two months later by imposing a nationwide ban on all agricultural uses of EDB (Fed. Regist., 1983). State environmental regulatory agencies of South Carolina, Washington, Arizona, Massachusetts, and Connecticut have subsequently reported EDB contamination of groundwater. The range of typical EDB concentrations in these states as well as California, Georgia, and Florida is 0.05 - 20 ppb (U.S. EPA, 1987b).

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[1]Aller, C. Bureau Chief, Groundwater Protection Division, Florida Department of Environmental Regulation, personal communication, March 12, 1985.

Ethylene Dibromide

Commercial Use

The EPA estimates that of the 750 to 790 thousand metric tons (one metric ton equals one thousand Kg) of EDB formerly produced annually in the United States, only 29 to 33 thousand metric tons were used in agriculture (U.S. EPA, 1983). The major industrial use was as an anti-knock lead-scavenging additive to leaded gasoline, constituting approximately 0.03% by weight of leaded gasoline. The major agricultural use (about 90%) was as a soil fumigant. Other registered agricultural uses included post-harvest fumigation, spot fumigation of grain milling machinery, and several other low volume uses (U.S. EPA, 1983). Its insecticidal and nematocidal properties were known in the mid-1920s. Federal pesticide registration for its use as a soil and grain fumigant was granted in the 1950s. EPA estimates that approximately one million acres in the U.S. were treated annually for nematode control with nearly 57 thousand metric tons of EDB. The largest portion of this was employed in farming of soybean, cotton and peanuts, with lesser amounts used in farming other crops. The greatest amounts of EDB have been used in the southeastern United States, where these crops predominate. EDB application increased somewhat when a closely related fumigant, DBCP, was banned in 1978.

Toxicity

The National Cancer Institute in 1973 first reported that EDB produces stomach cancer in rats and mice with a very short latent period (Olson et al., 1973). The chemical was mutagenic at relatively high doses (0.60, 0.092 revertants/nmol) in the Ames Salmonella reversion assay. The mutagenicity is not enhanced by addition of rat liver microsomal extract (McCann et al., 1975; Moriya et al., 1983). EDB induced lethal point mutations in fruit flies (Buselmaier et al., 1974) and was acutely toxic in all animals tested, with LD_{50} values ranging from 50 mg/Kg in rabbits to 450 mg/Kg in female mice (National Cancer Institute, 1978).

The toxic effects of EDB have been postulated to originate via either of its two known metabolic routes. A mixed function oxidase (P-450) mediated mechanism through the intermediate, bromoacetaldehyde, is followed by conjugation with glutathione (GSH) which is further metabolized to the water-soluble mercapturic derivative. The second route involves direct conjugation to GSH, to produce the half-mustard, S-2-bromoethyl-glutathione. This reaction is followed by formation of the highly reactive thiiranium ion, which may also be further metabolized to the mecapturic acid water-soluble form (van Bladeren et al., 1980; Shih and Hill, 1981). The later route has been implicated in producing the most profound toxic effects.

Genotoxic damage involves irreversible binding to DNA (Hill and Shih, 1978) and more specifically, as a S-[2-(N^7-Guanyl)ethyl]glutathione adduct (Inskeep et al., 1986). These reactions are dependent on cytosolic components and reduced glutathione (Ozawa and Guengerich, 1983; Inskeep and Guengerich, 1984).

Early indications of the toxicity of EDB prompted the Rebuttable Presumption Against Registration (RPAR) proceedings by EPA in December, 1977 (Fed. Regist., 1977). The EPA's final decision to suspend usage of this chemical was based in part upon the subsequent toxicologic findings and estimates of cancer risks associated with discoveries of EDB residues in the diet and drinking water. EPA's Cancer Assessment Group evaluated the increased cancer risk from EDB-contaminated drinking water using a one-hit model with "Weibull" timing (U.S. EPA, 1983, p 79). The resulting risks are shown in Table 1.

In one of two human epidemiological studies published, mortality data from workers exposed to the chemical in two EDB production plants were inconclusive in defining the relationship between EDB and human cancer (Ott et al., 1980). One notable conclusion, however, was that far fewer human neoplasms were observed than what was extrapolated from animal carcinogenicity studies (Ott et al., 1980). In the second study, a decrease in fertility of men occupationally exposed to EDB was significant in one of four workplaces studied (Wong et al., 1979).

Table 1. U.S. EPA estimates of cancer risk for EDB groundwater contamination.

CONCENTRATION of EDB, ppb (μg/L)	LIFETIME CANCER RISK[a]
0.02	0.00005
0.1	0.00015
5.0	0.0075
100.0	0.15

Source: U.S. EPA, 1983.

[a] Assumes consumption of 2 L of water per day, 60 Kg adult, for 70 years. These probabilities of observation of cancer are the additional risks estimated for a lifetime exposure.

Evaluating the evidence for EDB carcinogenicity, the International Agency for Research on Cancer and the American Medical Association find sufficient evidence from animal data but inadequate evidence for humans (O'Neill, 1985; Council on Scientific Affairs, 1988). EPA classifies EDB as a probable human carcinogen (group B2). Group B2 compounds are compounds with limited human epidemiologic studies and sufficient evidence from animal studies.

Chemical and Physical Properties

EDB is the saturated vicinal dibrominated product of ethylene. It is moderately soluble in water (4300 ppm or 22.9 mM at 30°C), compared to most pesticides and is fairly volatile (11.6 mm Hg, 1.5×10^{-2} atm at 20°C). Having a Henry's law constant (6.6×10^{-4} atm-m^3/mol) which is considered mid-range among commonly occurring chemical contaminants (Lyman, 1982a) combined with a relatively low affinity for the organic fraction of soil (organic carbon coefficient, 66 mg/g, as reported by Rogers and Mcfarlane, 1981; 44 mg/g, as cited in Lyman, 1982b), makes the chemical suspect to move freely through the underground compartment where it is introduced intentionally as a fumigant or unintentionally from leaking underground gasoline storage tanks.

Ethylene Dibromide in Florida Groundwater and Soil

The locations and levels of EDB in contaminated groundwater are related to dry and wet time periods and to

topography of the land. This pattern presumably reflects the halting and restarting of dilution of EDB in the aquifer. For example, a sharp rise in EDB content in the Floridian aquifer in the eastern part of the Florida panhandle paralleled the onset of the drought of mid-1986 in the southeastern United States. In one location studied, the proximity of sinkholes to areas of EDB application favored the contamination of underlying limestone aquifers.

As noted earlier, after the initial discoveries of EDB in groundwater in California, Georgia and Florida, at least five other states reported finding the compound in their groundwater. The situation in Florida, however, appeared the most severe.

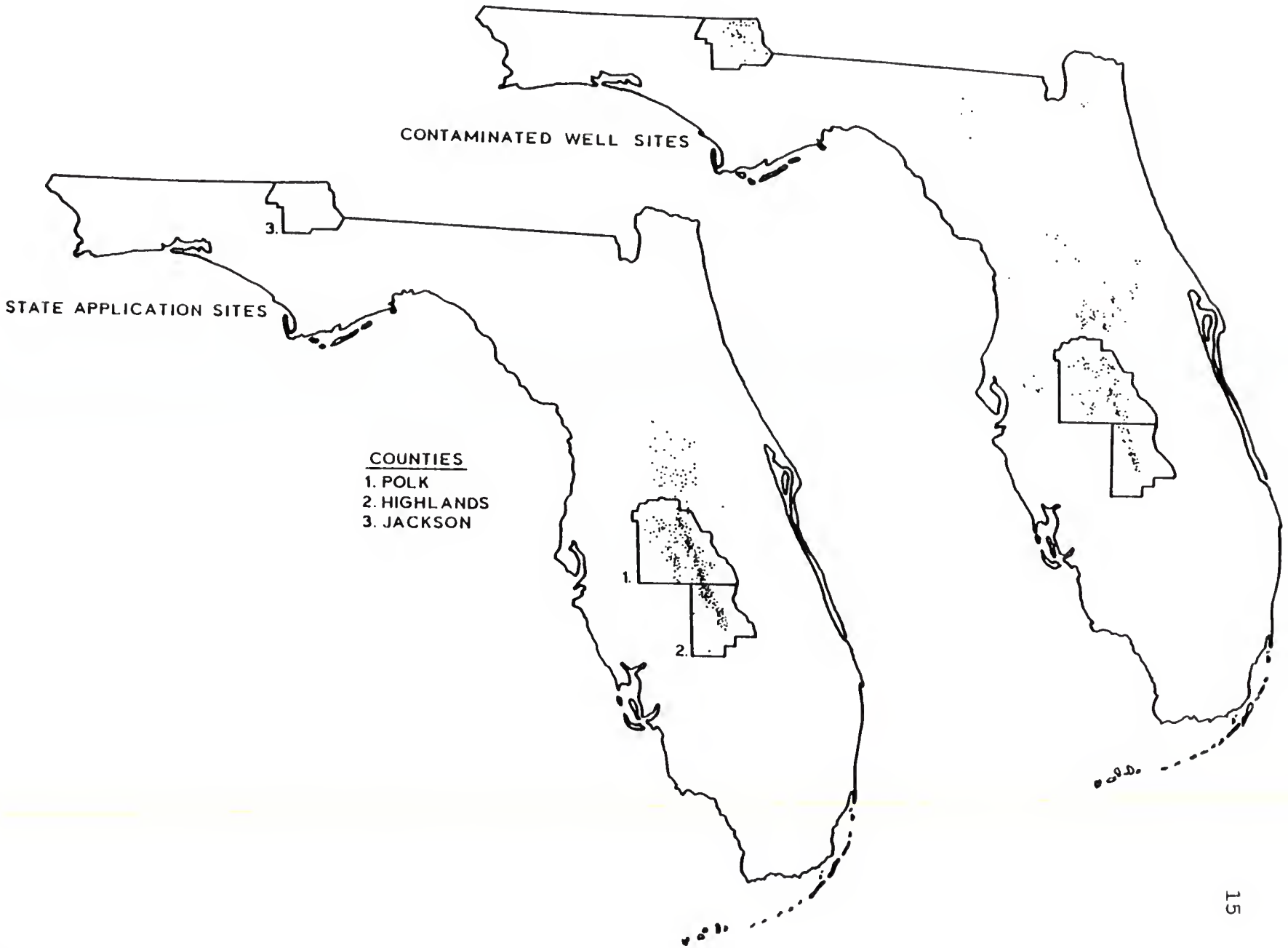
Responding to an early indication of the problem, the state of Florida assembled a multi-departmental task force headed by the Department of Environmental Regulation. Its goals were to oversee and implement a state-wide groundwater surveillance program, to gather available related information and initiate necessary research, and to offer assistance to residents of affected areas. Monitoring of public and private wells since June 1983 has shown that of the 67 counties in Florida, 22 counties had wells delivering water contaminated with EDB. Concentrations of EDB detected have ranged from 0.02 to about 900 ppb (Aller, C., personal communication, see footnote, p 6). Figure 1 represents the location of State EDB applications and well water sites

known to be contaminated with EDB as of March 1985. The EDB surveillance program database indicates that by June 1989, 1,703 wells of 13,441 tested (12.7%) were positive for EDB contamination. Approximately 86% of the positives found were in the counties of Highlands, Jackson, Polk, Lake, and Orange. With the exception of Jackson, most of the contamination (80 to 100%) resulted from the State nematode eradication program. Ninety percent of the wells tested in these counties are private wells. Less than 0.1% of wells tested had EDB concentrations greater than 10.00 ppb. The majority of the positives were between 0.01 and 1.00 ppb (Florida Groundwater Protection Task Force Meeting Minutes, 1989).

EDB has been used in Florida by horticultural and agricultural concerns and in golf course maintenance. Since EDB was not classified as a restricted use pesticide by the EPA, users were not required to maintain records. This lack of record keeping makes it difficult to predict areas at high risk for contamination and therefore difficult to make decisions about remediation of this contamination.

Starting in 1960, the Florida Department of Agriculture documented its use of EDB in their Spreading Decline Control Program. The disease known as spreading decline is the result of microbial infection of crops transmitted by the burrowing nematode. Practices included the establishment of barriers or buffer zones to prevent the

Figure 1. State of Florida nematode eradication program: application sites of EDB and EDB-contaminated wells as of March 1985.


CONTAMINATED WELL SITES
STATE APPLICATION SITES
COUNTIES
1. POLK
2. HIGHLANDS
3. JACKSON
1.
2.
3.

Feldman, 1965). State records indicate that the EDB application rate had been about 60,000 to 70,000 liters per year or an estimated total of 660,000 to 770,000 liters applied since initiation of the program. The application rate of EDB in this nematode eradication program was 25 to 50 lbs/acre[1]. Approximately 9600 cumulative acres were treated as barriers, which mainly consist of retreated land which average 417 acres treated per year. EDB was applied by injection at least eight inches beneath the soil surface through tubes behind coulter openers or chisels. The resulting furrows were closed by hilling soil over them, by press wheels or other means, to prevent loss of the EDB fumes from the soil (Florida Dept. Agric., 1984). State-sponsored application of EDB correlates well with cumulative contamination data, as shown in Figure 1, with the exception of the northwestern region of the State. The use of EDB in soil by the State was estimated to be only about 10 percent of all the EDB applied. Records of non-State-sponsored applications are scant, making reliable estimates of usage impossible (Shankland, 1985). Generally, contaminated areas are consistent with records compiled by inspectors from information volunteered by agricultural growers for the last two years of EDB application around the State, with at least

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[1]Written correspondence dated May 9, 1984 from D.Conner, Florida Secretary of Agriculture, responding to the information requested by Florida Senator P. K. Neal, regarding State EDB soil application.

one notable exception. Records from Dade county in south Florida indicate that EDB had been applied in the greatest quantity, yet no contamination has been found. There is no explanation for this observation; however, more rapid groundwater flow compared to other areas of the State may have contributed to faster dilution of the chemical.

Sorption to Soil

Despite the dispersal of significant amounts of EDB into the environment from pesticide application, emissions from gasoline-engine combustion and leakage of underground tanks, little was known about the persistence, fate and transport of the compound in the natural environment, especially, in soil and water. Values as divergent as five to 10 days (Johns, 1976; Leinster et al., 1978) and 14 years (National Institute of Occupational Safety and Health, 1977) were cited as half-lives due to hydrolysis in neutral aqueous solutions at ambient temperature. Early investigations focused primarily on residual EDB on crops and on processed or stored agricultural products (Heuser, 1961; Sinclair et al., 1962; Beckman et al., 1967).

The diffusability of EDB through soil is a crucial factor affecting its ability to reach and destroy soil-borne pests. An important factor that influences the diffusion is the adsorptive capacity of the soil for the fumigant vapor. Early work indicated that there is little penetration into soil mass above the point of injection of undisturbed soil

after treatment. Sorption was found to be primarily due to colloidal organic matter and therefore affected greatly by the amount of organic content of the soil (Siegal and Erickson, 1953). Sorption was rapid with very little, if any, irreversible sorption. A sharp drop in sorption followed small increases in moisture content of soils (i.e., from 15 to 17% moisture) with sorption leveling at about 25% soil moisture content. Temperature decreased the amount of EDB sorbed on soils by about 5% per degree between 10 and 25°C (Wade, 1954). The sorption capacity of soil was closely related to the observed concentration ratio in the soil-water phase to the vapor phase (Henry's law constant), with a three-fold decrease in sorption accompanying a temperature increase from 5 to 25° C (Thomason et al., 1974). For up to twenty days after simple line injection, there was very little loss of EDB to the atmosphere through an undisturbed soil profile. A loss of less than 5% of EDB was measured after a chisel shank hole injection at a commonly employed depth of 30.5 cm, during which time a constant air stream at a rate of 0.80 Km/hr was passing over the surface of the soil.

In contrast, investigators fit their experimental data to the Brunauer, Emmett, and Teller (BET) multi-molecular isotherm adsorption equation. They found that EDB sorption potentials to soils were essentially restricted to the external surface of the soil particle and not organic

material (Jurinak and Volman, 1957). The BET adsorption theory is based primarily on the assumption that Van der Waal's forces between molecules play the predominant role in adsorption after the mono-layer is formed. The ability of EDB to penetrate water structure in close proximity to soil surfaces is greatly inhibited by increased moisture content (Call, 1957). The competition between water and EDB for sites on soil surfaces is also a function of soil type (Phillips, 1964).

Under field conditions, two or more interacting edaphic factors in soil were found to determine the overall diffusion behavior. Penetration in coarse soil was largely dependent upon moisture content and temperature; medium-textured soil penetration was dependent upon moisture, temperature and bulk density; no movement occurred in fine-textured soil (Townsend et al., 1980). In closely related studies, sorption of neutral organic compounds was shown to be essentially a partitioning-controlled process rather than a physical adsorption process (Choiu and Peters, 1979; Rogers and McFarlane, 1981).

Degradation in Soil and Water

Evaluation of the ability of microbes to degrade EDB has been reported by two laboratories. Bacterial batch cultures of primary sewage effluent incubated under anoxic denitrifying conditions failed to degrade EDB (Bouwer and McCarty, 1983). It was found, however, to be rapidly

transformed under anoxic methanogenic conditions in batch cultures and in a continuous-flow methanogenic fixed-film column (Bouwer and McCarty, 1985). Anaerobic suspensions of sewage sludge, activated by percolating oxygen through the sewage, gave similar findings. The gaseous product in this case was confirmed to be ethylene (Weintraub et al., 1986). This laboratory also found that similar suspensions incubated under aerobic conditions resulted in rapid degradation to a non-volatile, debrominated and highly water-soluble product (Jex et al., 1985). Separation of this product was achieved by high performance liquid and thin layer chromatography plus chemical derivatization for gas chromatography and mass spectrometry analysis. Analytical results suggested a single product which probably is a condensation or complexation with organic materials or microbial cells.

The ability of natural microbiota to degrade EDB from contaminated areas has been reported by two laboratories. In the first study, soil was obtained from three Florida farming regions where the chemical was used extensively for many years. Soil samples from depths of one and three meters, which were assumed to be anaerobic, were incubated as such in aqueous suspensions. Only inorganic nutrients were added and incubated for seven months. The investigators found that EDB was either not degraded or only marginally degraded by organisms in Florida soil (Weintraub

et al., 1986). In contrast, the second study found EDB to be rapidly mineralized to carbon dioxide and bromide ion by organisms in surface soils under aerobic incubation conditions. Soil and water were sampled from an EDB-contaminated groundwater discharge area in Connecticut. The conversion of EDB to these products was almost complete with the exception of only small amounts of an unidentified unextractable product (Pignatello, 1987).

Wilson et al. (1986) found that EDB was rapidly degraded in aquifer material known to support methanogenesis. The equilibrium redox potential of the material used was -272 mV. Concentrations of 200 ppb of EDB in the aquifer matter-pore water used declined to 27% of the control concentration by seven weeks of incubation at 17°C. No carbon dioxide was detected as a degradation product. Although no product was identified, a peak observed by gas chromatography with flame ionization detection coincided with the disappearance of EDB; subsequent GC/MS was not successful in identifying this peak. The report made no mention of the presence of hydrogen sulfide, commonly present in the material described and possibly a very important reactant in the incubation material.

Steinberg, et al. (1987) have reported residual EDB in soils from Connecticut farmlands highly resistant to degradation or mobilization. The EDB was released with heat and organic solvent treatments. Central Florida soils that

were treated approximately bi-annually, but had not received EDB treatment for at least 5 years, had EDB residues up to 60 ng/g of dry soil (Moye and Weintraub, 1988). The EDB in these soils was released with 75°C acetonitrile incubation. About 1 ngEDB/g dry soil was released from some of the soils after exposure to 45°C dry heat for 8 hr followed by and aqueous washing. The importance of a fraction of "soil-bound" EDB in contaminated areas is still being evaluated.

Other modes of degradation of EDB have also been investigated recently. EDB in aqueous solution is rapidly photohydrolyzed upon irradiation with a 450-W medium pressure mercury lamp to yield ethylene glycol and bromide ion. The rate enhancement for this process was estimated to be 10^5 times greater than that of the nonphotolytic pathway (Castro and Besler, 1985). In another study, EDB was shown to undergo complete heterogeneous photocatalyzed transformation to carbon dioxide and hydrogen bromide (Nguyen and Ollis, 1984). This conversion was catalyzed on titanium dioxide when illuminated with near-UV radiation. The titanium dioxide surface is suggested as the site of formation of hydroxyl radicals which promote the reaction. Other investigators have found that the superoxide ion dioxygenates EDB to yield formaldehyde and bromide ion in aprotic solvents (Calderwood and Sawyer, 1984). The later three modes of degradation of EDB have been suggested by the authors as useful ways to dispose of halogenated pesticide

wastes such as EDB. Finally, oxidation of EDB to formaldehyde in aqueous solution has been reported to occur at high temperatures (i.e. greater than 80°C). The conversion, however, was not found to be significant under environmental conditions. In this same laboratory, EDB at concentrations of 300 and 700 ppb was found to be stable over a two-week period at 25°C in solutions of aqueous chlorine which are commonly attained during disinfectant processes in municipal water facilities. Total concentrations of one and 10 ppm of the two species, hypochlorous acid (HOCl) and hypochlorite ion ($^{-}$OCl), were varied by buffering the solutions from pH 6.5 to 9 (Moye et al., 1986). It is important to note that the above mentioned studies are laboratory modes of reaction that do not necessarily reflect reactions in the field.

An additional route of degradation of EDB in the environment may have been uncovered by recent work by Schwarzenbach et al. (1985). These investigators detected numerous dialkyl sulfides and other volatile sulfur-containing compounds while sampling wells in an area contaminated seven years earlier by commercially produced volatile halogenated hydrocarbons. In laboratory studies it was revealed that at least for a representative test compound, 1-bromohexane, under highly reducing conditions in the presence of hydrogen sulfide, nucleophilic substitution reactions proceed quite readily. A recent report by Watts

and Brown (1986) describing the findings of organics in contaminated well fields in Palm Beach county, Florida, includes the sulfur-containing compounds ethyl mercaptan, triethyl disulfide, and diethyl disulfide, as well as bromoethane. Although the components of gasoline were also found in the well water, no EDB was reported. The possibility of a similar reaction occurring with EDB in anaerobic groundwater has recently been reported by Weintraub and Moye (1987).

Vogel and Reinhard (1986) identify the reductive dehalogenation product of EDB, vinyl bromide, as a major product in their hydrolysis study of EDB. If, in fact, vinyl bromide (Moye and Weintraub, 1986), is the major product, than EDB's degradation in groundwater does not decrease toxicity (Henschler, 1985).

Summaries of the modes of degradation of EDB discussed here are presented in Tables 2 and 3. Ongoing and future work may enable scientists to understand the variations and relative importance of these and possibly other modes of degradation in relation to the persistence and hazard presented by this chemical in the environment.

Table 2. Summary of microbial transformation of EDB.

CONDITIONS	HALF-LIFE	PRODUCTS	REFERENCE
Field crops	"rapid"	Br^-, ?	Beckmann et al. (1968)
Soil-water cultures	ca. 30 d	Br- + ethylene	Castro, Besler (1968)
Anaerobic (denitrifying)	N.D.	---	Bouwer, McCarty (1983)
Anaerobic (methanogenic)	---	Br- + ethylene	Bouwer, McCarty (1985)
Florida soils (methanogenic)	N.D.	---	Weintraub et al. (1986)
Activated-sludge (methanogenic)	ca. 21 d	Br- + ethylene	Jex et al. (1985)
Activated-sludge (aerobic)	5-10 d	Br-, Biomass	Jex et al. (1985)
CT stream-sediment (aerobic)	35-350 d	Br-, CO_2, biomass	Pignatello (1986)
Aquifer material (methanogenic)	ca. 21 d	Unidentified	Wilson et al. (1986)

N.D. = None observed

Table 3. Summary of chemical transformations of EDB.

MODE	CONDITIONS	HALF-LIFE	PRODUCTS	REFERENCE
HYDROLYSIS	Neutral, aq 25°C	5-10 d	Not Reported	John (1986)
	Neutral, aq 20°C	14 yr	Not Reported	NIOSH (1977)
	Neutral, 'Zero buffer' 20°C	8 yr	Br^-, Unident-fied	Cohen,Jungclaus (1986)
	FL groundwater abiotic, 22°C	1.5-2 yr	Ethylene glycol + Br^-	Weintraub et al. (1986)
PHOTOLYSIS	UV, aq	10 d	Ethylene glycol + Br^-	Castro et al. (1985)
SULFIDE SUBSTITUTION	Buffer-aq, 5-75 ppm H_2S, 25°C	2-4 mo.	Cyclic-alkyl and dialkyl- sulfides	Weintraub et al. (1987)
REDUCTIVE DEHALOGENATION	Aq, 20°C	2.5 yr	vinyl bromide + Unidentified	Vogel, Reinhard (1986)

Risk Factors for Groundwater Contamination

Florida's citrus, soybean and peanut farming areas, where EDB was commonly used, are located on predominantly sandy and well-drained soils with very low organic matter content (i.e., less than 1%). The soils are mostly entisol, spodosol, alfisol and ultisol types. Together with a relatively high mean annual rainfall and high recharge to the Floridian aquifer in these regions (Fernald and Patton, 1984), the potential for movement of certain chemicals through the unsaturated subsurface zone is great. As previously explained, EDB has this ability to be quite mobile as well as persistent.

There have been a number of attempts in recent years to develop comprehensive groundwater protection strategies. The main goal has been to establish criteria upon which to base regulatory and monitoring decisions to guard against groundwater contamination. The EPA began such an effort in 1979 to identify pesticide characteristics, nitrate levels, and soil pH that would likely result in groundwater contamination (Cohen et al., 1984). Another approach currently proposed by the EPA makes use of a numerical rating scheme for evaluating the potential for groundwater contamination in a specific site given its geohydrological setting. The scheme is called DRASTIC, an acronym for (i) depth to groundwater, (ii) recharge rate, (iii) aquifer media, (iv) soil media, (v) topography, (vi) impact of the

vadous zone, and (vii) conductivity of the aquifer (Rao et al., 1985).

More recently, researchers at the University of Florida have developed a simple scheme for ranking the relative potentials of different pesticides to intrude into groundwater (Rao et al., 1985). The index, referred to as the attenuation factor (AF), is intended for use by regulatory agencies in a preliminary evaluation of a large number of pesticides to target chemicals for groundwater monitoring programs or to initiate site-specific studies. Of the 40 chemicals evaluated, the top 5 ranking pesticides were EDB, bromacil, picloram, DBCP, and diuron. Not surprisingly, EDB ranked as having the greatest potential for groundwater intrusion in the soils tested.

MATERIALS AND METHODS

Materials

The following is a list of materials used in this study along with the supplier and stated purity or grade when provided by the manufacturer. The supplier's location is listed only the first time it is mentioned.

Analytical Standards

Bromoacetic acid (Aldrich Chemical: Milwaukee, WI; 98%)

Bromoethane (Aldrich Chemical; 99^{+}%)

2-Bromoethanol (Eastman Kodak; Rochester, NY 98%)

1-Butanethiol (Eastman Kodak; 99^{+}%)

Butyldisulfide (Aldrich Chemical: Milwaukee, WI; 98%)

Cyclohexylthiol (Aldrich Chemical; 95^{+}%)

1,2-Dibromoethane (Aldrich Chemical; Gold Label; 99^{+}%)

1,2-Dibromoethane (ethylene dibromide, EDB; EPA Pesticides and Industrial Chemical Repository: Research Triangle Park, NC; 99^{+}%)

1,4-Dithiane (Aldrich Chemical; 97%)

1,3-Dithiolane-2-thione (Aldrich Chemical; 99%)

1,2-Ethanedithiol (Eastman Kodak; 99.5%)

Ethanethiol (Eastman Kodak; 98%)

Ethyl disulfide (Aldrich Chemical; 99%)

Ethylene glycol (Eastman Kodak; 99^{+}%)

37% Formaldehyde solution (Fisher Scientific: Fair Lawn, NJ; ACS Certified, 36.6%)

Paraformaldehyde (Fisher Scientific; 95%)

Potassium bromide (Mallinckrodt: Paris, NY; analytical grade)

1,4,7,10-Tetrathiacyclododecane (Aldrich Chemical: West Chester; 97%)

1,4,7-Trithiacyclononane (Aldrich Chemical; 98%)

1,3,5-Trithiane (Chem Service: West Chester; 99%)

Vinyl bromide (Aldrich; 98%)

Radiolabeled Chemicals

^{14}C-Formaldehyde (4.4 mCi/mmoL, Pathfinders: St. Louis, MO; 98%)

U-^{14}C-Toluene (1.8 μCi/mL, New England Nuclear: Boston, MA)

U-^{14}C-Ethylene dibromide (55 mCi/mmoL, Amersham: Arlington Heights, IL; 95^{+}%)

U-^{14}C-Ethylene dibromide (U-25 mCi/mmoL, Amersham; 95^{+}%)

U-^{14}C-Ethylene glycol (U-4.23 mCi/mmoL, Pathfinders; 98%)

Solvents and Reagents

Aqua-Sol 2 (New England Nuclear)

Buffer solutions, pH 4, 7, 10 (Fisher Scientific; ACS Certified)

Chloroamine T (Fisher Scientific; ACS Certified)

Chloroform (Fisher Scientific; HPLC-grade)

Chromotropic acid disodium (Fisher Scientific; ACS Certified)

N,N-Dimethyl-p-phenylenediamine dihydrochloride (Aldrich Chemical; 99%)

Ferric chloride hexahydrate (Aldrich Chemical; 97%)

Ferric ammonium sulfate (Fisher Scientific, ACS Certified)

Hexanes (Fisher Scientific; pesticide grade)

Iodine (Fisher Scientific; Certified ACS)

2-Nitrophenylhydrazine (ICN Pharmaceuticals, Plainview, NY)

Periodic acid (Mallinckrodt; ACS analytical grade)

Potassium nitrate (Fisher Scientific; Certified ACS)

Potassium fluoride (Fisher Scientific; Certified ACS)

Potassium ferrocyanide (Fisher Scientific; Certified)

Potassium ferricyanide (Fisher Scientific; Certified ACS)

ScintiVerse II (Fisher Scientific)

Sodium sulfide, 9-hydrate (Eastman Kodak; ACS reagent, 98.0-103.0%)

Sodium sulfite (Fisher Scientific; Certified ACS)

Sulfuric acid (Fisher Scientific; Certified ACS)

Triethylamine (Aldrich Chemical; 97%)

Water (Fisher Scientific; HPLC-grade)

Components of Buffers

All of the following chemicals were Fisher Scientific, ACS Certified grade unless otherwise indicated.

Borax (sodium tetraborate, Sigma Chemical: St. Louis, MO; 99.9%)

Disodium dihydrogen phosphate hepta hydrate

Disodium borate decahydrate

Disodium carbonate

Glacial acetic acid (reagent ACS)

Hydrochloric acid (reagent ACS)

Monopotassium phosphate

Potassium acetate

Potassium phthalate

Potassium chloride

Sodium monohydrogen phosphate monohydrate

Sodium citrate

Sodium hydroxide

Sodium monohydrogen carbonate

Sodium borate

Sodium carbonate decahydrate

Sodium succinate

Titanium trichloride solution (20%/80% water stabilized with 3% phosphoric acid)

Gas Chromatographic Materials and Columns

5% Carbowax 20M (Supelco: Bellefonte, PA)

Gas Chrom Q 80/100 mesh (Supelco)

15% OV-17 on Gas-Chrom Q (Alltech Assoc.: Deerfield, IL)

4% OV-225 on Chromosorb Q (Supelco)

Phenylmethylsilicone crosslinked coated capillary column (DB-5, J & W Scientific: Folsom, CA)

Polymethylsiloxane crosslinked coated capillary column (Ultra 1, Hewlett-Packard: Palo Alto, CA)

Polyphenylmethylsiloxane coated wide bore column (RSL-300, Alltech Assoc.)

Polypropylene glycol (Supelco)

10% Squalane on Chromosorb W (Supelco)

Methods

Ethylene Dibromide and Product Identification and Quantitation by Gas Chromatography and Spectrophotometry

Gas chromatography of EDB and related brominated metabolites

Gas chromatograhpic (GC) conditions were established for the simultaneous analysis EDB and other closely related bromominated metabolites (bromoethane, bromoacetic acid and 2-bromoethanol) on a Hewlett-Packard Model 5840A gas chromatograph (GC) equipped with a ^{63}Ni electron capture detector (EC) and a Hewlett-Packard Model 7671 A autoinjector. The 2 m x 0.32 cm i.d. glass column used was

packed with 15% polypropylene glycol (PPG) on Gas Chrom Q 80/100 mesh solid support which was coated in this laboratory. The solid support was coated by slow rotoevaporation of 50 mL of acetonitrile in which the 3 g of PPG was dissolved and 17 g of solid support was added. The resulting stationary phase achieved superior separation of the analytes compared to others tested. The column packings which were used perviously EDB and similar analytes and tested include 5% Carbowax 20M (polyethylene glycol, Dumas and Bond, 1982), 15% OV-17 (Rains and Holder, 1981), 4% OV-225, and 10% squalane. The PPG was used for the determination of ethylene chrorohydrin (2-chloroehtanol) by Heuser and Scudamore (1967).

The PPG column prepared was used with a 5% methane/95% Ar carrier gas at a flow of 52 mL/min. Temperatures maintained during analysis were 100°C for the column, 300°C for the detector and 225°C for the injector.

EDB was extracted from aqueous solutions by partitioning into hexane upon mixing. For experiments in which solutions were incubated in 100 mL serum bottles, the following procedures were followed. Using a 10 mL syringe a 10 ml aliquot was withdrawn through the Teflon-coated rubber septa, and slowly delivered to a 10 mL volumetric flask to assure a precise volume. This volume was transferred to a 15 mL screw-top test tube to which 1.0 mL of hexane was added and the tube immediately capped. It was inverted 5

more times and allowed to stand for at least 5 min. A portion of the hexane layer was pipetted into a 1 mL vial for automated gas chromatographic analysis. In subsequent experiments where solutions were incubated in 10 mL sealed ampules or where the partitioning of ^{14}C-activity was analyzed, 2 mL of soltions were transferred with a tranfer pipet to test tubes containing 2.0 mL of hexane. All extractions of aqueous samples were performed in duplicate. Extracts were injected in duplicate into the GC. Standard curves of detector response ranging from 20 to 1500 pg/μL of EDB were prepared daily and used for quantitation using linear regression least squares analysis.

Determination of ethylene glycol

Ethylene glycol concentrations in water were determined by GC analysis of the 2-nitrophenylhydrazine derivative of formaldehyde, formyl 2-nitrophenylhydrazone. The method of Fishkind (1987) for the analysis of formaldehyde in water was adapted by including a preliminary oxidation step. To 2 or 4 mL of the water sample, a 20 μL aliquot of 5 x 10^{-6} M periodate and the sample was then allowed to stand at room temperature in the dark for 20 hours. Periodic acid oxidizes ethylene glycol into two molecules of formaldehyde (March, 1985). Excess periodic acid was precipitated by the addition of 50 μL of a saturated solution of potassium nitrate; the mixture was allowed to stand for 1 hr in an ice bath (Hillenbrand, 1952).

Standard aqueous solutions of ethylene glycol prepared from analytical grade ethylene glycol were assayed by the same procedure each day of analysis to construct a standard curve for quantitation. Aqueous formaldehyde, which was either formed by the oxidation of ethylene glycol or was present in standard solutions, was quantitated by the addition of 640 μL of a 2 M aqueous solution of 2-nitrophenylhydrazine, which had been recrystallized from hexane to remove background formaldehyde. After the addition of this derivatizing reagent, the reaction mixture was incubated in a water bath at 40°C for 1 hr. The derivative 2-nitrophenylhydrazone was extracted by the addition of 5 mL of hexane to the tube, which was then mixed on a vortex-mixer for 1 min. A portion of the hexane layer was pipetted into a 1 mL vial for automated analysis on a Hewlett-Packard Model 5890 GC. This instrument was equipped with a Hewlett-Packard Model 7671 A autosampler, a ^{63}Ni electron capture detector, and a 2 m x 0.32 column packed with 4% OV-225 on Chromosorb Q 80/100 mesh. A 5% methane/95% Ar carrier gas at 30 mL/min, a column temperature of 173°C, a detector temperature of 300°C, and an injector temperature of 225°C were employed.

The method was validated with use of ^{14}C-ethylene glycol (specific acitivity 4.23 mCi/mmol) and ^{14}C-formaldehyde (specific activity 4.4 mCi/mmol). Unlabeled stock standard formaldehyde solutions made from para-

stock standard formaldehyde solutions made from paraformaldehyde were standardized against accurately made solutions of bisulfite formaldehyde and 37% formaldehyde solution using the chromatropic acid spectrophotometric method of Houle et al. (1970). Other supporting data used for optimization of these procedures are described by Fishkind (1987).

Determination of bromide

Bromide ion concentrations in water were analyzed by Standard Water and Watsewater Methods Procedure No. 405 C (American Public Health Association, 1980, p 261). Bromide ion was oxidized by Chloroamine T to bromine which brominates phenol red. The brominated product was measured by absorbance of the reaction mixture at 590 nm on a Beckman DU-8 spectrophotometer. Standard aqueous solutions of bromide prepared from potassium bromide were assayed to construct a standard curve over a range of 50 to 1000 μg/L. Samples were diluted as needed with deionized water to final concentrations which fell within the linear quanitative range.

Liquid Scintillation Counting of ^{14}C-ethylene Dibromide

Total ^{14}C-activity was measured by liquid scintillation counting of 1.0 mL of sample pipetted into 15 mL of liquid scintillation fluid. Either Aqua-Sol 2 or Scinti Verse II liquid scintillation fluids were used. There was no difference in results using either of these reagents.

Counting was done on a Searle Analytical 92 liquid scintillation counter with a Silent 700 electronic data terminal.

Quenching was evaluated both by comparing additions of the fortified waters or hexane extracts to additions of known amounts of ^{14}C-toluene ($4x10^6$ dpm/mL) and by external addition of 20 μL of ^{14}C-toluene to fortified waters or hexane extracts. The dpm/mL in aqueous solutions or hexane extracts was determined by dividing the measured cpm/min by the counting efficency. Efficiency and precision of extraction of EDB from the waters were evaluated in a similar fashion.

Gas Chromatography and Mass Spectrometry for Sulfur Product Identification

Analyses were performed on a GC with a flame photometric detector with a 394 nm filter for sulfur-containing compound selectivity (FPD/S). With this filter, the predominant chemiluminesence bands of emmision of sulfur-containing analytes are detected (Farwell and Barinaga, 1986). Analyses were performed on a 5840A Hewlett Packard GC equipped with a Hewlett Packard 7671A autosampler and a 15 M x 0.53 mm i.d. wide bore column with 1.2 μm polyphenylmethylsiloxane film (RSL-300). The oven temperature was programmed as follows: it was held at 40°C for 2 min, then increased at a rate of 8°C/min to 90°C, then 25°C/min to 200°C. The carrier gas flow of nitrogen at 10 mL/min. FPD/S conditions were as follows: injector

temperature 170°C, detector temperature 225°C, hydrogen 80 mL/min, oxygen 14 mL/min and air 55 mL/min.

Extracts from aqueous incubations, usually a 5:1 ratio water to hexane, and the sulfur-containing standards in hexane also were analyzed by GC/MS on a Finnigan 4021 GC/MS. This instrument was equipped with a 30 M x 0.25 mm i.d., 0.33 μm phenylmethylsilicone crosslinked film coated capillary column (DB-5). The GC oven temperature was programmed to begin at 60°C and increase at a rate of 12°C/min to 280°C. Mass spectra were obtained in electron impact (EI) and chemical ionization (CI) modes, both at 70 eV and a source temperature of 150°C. The CI spectra were collected with isobutane reagent gas at 0.2 torr.

Oxygen, Redox, and pH Measurements

Dissolved oxygen was measured with a membrane oxygen electrode (model 97-08, Orion Research, Cambridge, MA). The electrode was calibrated in water-saturated air and zeroed in a 5% sodium sulfite solution. Measurements were made in a 100 mL BOD bottle without headspace.

Redox potential (E_h) was measured with a platinum redox electrode (model 97-80, Orion Research Inc., Cambridge, MA). This electrode was calibrated using the following solutions: (1) 0.1 M potassium ferrocyanide with 0.05 M potassium ferricyanide, (2) 0.01 M potassium ferrocyanide, 0.05 M potassium ferricyanide, and 0.36 M potassium fluoride, and (3) a standard ferrous-ferric solution composed of 0.1 M

ferrous ammonium sulfate, 0.1 M ferric ammonium sulfate, and 0.1 M sulfuric acid (Light, 1972).

The pH was measured with a glass body combination electrode (model EA-2, Fisher Scientific, Fair Lawn, NJ) and calibrated in standard pH 4, 7, and 10 buffer electrode standardization solutions. All of the above mentioned electrodes were connected to an Orion model 601A digital ionanalyzer for operation.

Hydrolysis Reactions

Kinetics: Effects of Temperature and pH

Preparation and incubation of solutions

Groundwater was obtained from shallow wells in three counties in northcentral and northwestern regions of the State: Polk, Highlands, and Jackson. Water samples were characterized by analyses either on-site, at this laboratory, or at the Extension Soil Testing and Analytical Research Laboratory, University of Florida (Appendix A). The water was collected in five-gallon polypropylene containers and kept on ice during transport to this laboratory. Water was assayed for residual EDB by the packed columm GC analysis.

One hundred ml aliquots of these water samples and of laboratory deionized water were fortified with EDB to concentrations of 10 or 100 ppb with a methanol/water stock solution of the chemical. Glass serum bottles (100 mL,

Wheaton, Millville, NJ) were filled with the solutions, the headspace purged with nitrogen and the bottles tightly capped with Teflon-coated rubber septum seal crimp caps (Supelco, Bellefonte, PA). Before sample preparation, the serum bottles, seals, and caps were autoclaved (121°C, 15 psi, 20 min) and the waters were either autoclaved (same conditions) or vacuum-filtered through a 0.20 μm filter (Millipore, Bedford, MA) to eliminate microbial activity.

Sets of samples prepared in duplicate were incubated in an inverted position in a darkened water bath. Since EDB was relatively stable in water at ambient temperatures in preliminary experiments, incubations were performed at 40, 50, 60, 70 and 80°C ± 0.5°C. Serum bottles were periodically taken out of the water bath and allowed to equilibrate to room temperature before sampling. The time interval between samplings was determined by the observed rate of disappearance of EDB.

The apparent first-order rate constants for EDB degradation were obtained by linear regression least squares analysis of the log percent EDB remaining vs time plot. Arrhenius temperature dependence was assumed, whereby a plot of log rate constant vs 1/T °K provides a convenient graphic method to evaluate the activation energy, E_a, and to estimate the rate constant of the reaction at any desired temperature.

At least two samples in each incubation trial were fortified with a U-^{14}C-EDB (25 mCi/mmol) by addition from a

by addition from a 5 μCi/mL methanolic stock solution to a final concentration of ca. 5000 dpm/mL. These samples were periodically sampled to check the integrity of the system by measuring the containment of the ^{14}C-activity. The radiochemical purity was reported to be 98% (Amersham) and confirmed in this laboratory to be within 5% of the stated value as measured by gas chromatography and liquid scintillation counting. To check the efficiency of hexane extraction of EDB and its losses during the analysis procedure, solutions of ^{14}C-EDB were analyzed by identical procedures.

The degradation of EDB was evaluated as a function of pH in the three groundwaters and deionized water. A series of 5 mM buffer systems (Diem, 1962) were used to maintain groundwater and deionized water fortified with EDB at pH values ranging from 4.0 to 9.0. The buffers and concentrations of the components added for the resulting pH as indicated were as follows: borax/succinic acid: 0.89 mM/4.11 mM pH 4.0, 1.98 mM/3.02 mM pH 5.2; potassium phthalate/NaOH: 5.00 mM/0.04 mM pH 4.0, 5.00 mM/0.12 mM pH 4.6; borate/KCl/NaOH: 5.00 mM/5.00 mM/0.40 pH 8.0, 5.00 mM/5.00 mM/0.87 mM pH 9.0; borax/monopotassium phosphate: 0.28 mM/4.50 mM pH 8.0, 4.50 mM/0.13 mM pH 9.0; and sodium carbonate/disodium bicarbonate: 0.40 mM/4.26 mM pH 9.2, 4.60 mM/0.76 mM pH 10.6).

Incubations of the EDB-fortified buffered solutions were conducted at 62°C. The remaining EDB at approximately

3 day intervals was determined by hexane extraction and GC analysis on the PPG packed column. Rate constants for EDB degradation were derived from data collected over 17 days.

Product Identification

Polk, Highlands, and Jackson County groundwater samples and deionized water were fortified with EDB to a concentration ca. 3.5 mg/L, including ^{14}C-EDB with a resulting concentration of ca. 3500 dpm/mL. Incubations were conducted at 73°C until essentially all the EDB had disappeared. At approximately 2 day intervals, analyses of EDB, ethylene glycol, and bromide ion were performed. In addition, samples were analyzed for purgeables by gas chromatography/mass spectrometry (GC/MS). Within a purge and trap device (Tekmar model ALS, Cincinatti, OH), helium gas was bubbled at 8 mL/min through a 25 mL aliquot of a sample for 22 min at ambient temperature and the effluent directed onto Tenax adsorbent. The Tenax adsorbent was heated to direct the purgeables into a GC, with a PPG column and GC onditions used for EDB analysis. The GC was interfaced with a Finnigan Model 4021 quadrupole GC/MS for analysis. Mass spectra, scanning from m/e 50 to m/e 400, were obtained in electron impact mode with an ionization energy of 70 eV.

To measure the partitioning of ^{14}C-activity of the initial ^{14}C-EDB and its ^{14}C-products, 1 mL aliquots of the hexane and aqueous phases, after extraction, were pipetted into 15 mL of scintillation liquid and counted. In

addition, 1 mL of the unextracted sample was analyzed in the same manner.

Reactions with Aqueous Hydrogen Sulfide

Kinetics: Temperature and pH Dependence

Preparation of solutions

Experimental solutions were in 10 mM buffers prepared with HPLC grade water. Before use, glassware and water were autoclaved (121 °C, 15 psi, 20 min). The buffers providing solutions of pH 5, 7 and 9 consisted of the following components: potassium acetate/acetic acid: 6.40 mM/0.360 mM pH 5.0, potassium phthalate/sodium hydroxide: 2.36 mM/7.64 mM pH 5.0, sodium monohydrogen carbonate/hydrochloric acid: 8.17 mM/1.83 mM pH 7.0, disodium dihydrogen phosphate heptahydrate/sodium monohydrogen phosphate monohydrate: 3.81 mM/6.19 mM pH 7.0, disodium borate decahydrate/hydrochloric acid: 3.65/6.35 mM pH 9.0, disodium carbonate decahydrate/hydrochloric acid: 0.53 mM/9.47 mM pH 9.0). The resulting calculated ionic strengths of the solutions were adjusted to μ = 15 mM using 4.8 M KCl. The solutions were deoxygenated by bubbling a stream of oxygen-free nitrogen through them for at least 2 hr. The redox buffer, Titanium (III) citrate of Zehnder and Wahrmann (1976), was used in the experimental solutions to maintain an oxygen-free water phase with high electron activity to inhibit oxidation of sulfides. The redox buffer stock solution was perpared by adding 3.5 mL of Titanium trichloride solution (20%/80%

water) to a final volume of 50 mL made up in 0.2 M sodium citrate. One mL of the redox buffer stock solution was added to each 99 mL of experimental preparation.

All subsequent manipulations of solutions and filling of sample containers were done in an AtmosBag (Aldrich Chemical) which contained an nitrogen atmosphere. The oxygen concentration inside the Atmosbag remained below 0.05 mg/L. Oxygen concentration was monitored with an oxygen sensor (Bio-Tek Instruments, Inc., Winooski, VT, Model 74223).

Five hundred mL portions of the six buffer solutions were fortified with methanolic stock solutions of 26.5 mM EDB and 500 μCi/mL of ^{14}C-EDB (specific activity 55 mCi/mmol) to achieve final concentrations of 6.0 μM ca. EDB and 5000 dpm/mL. Crystals of disodium sulfide nonane hydrate were washed with deionized water and suction dried on filter paper before being weighed and dissolved in the buffered solutions. The final concentrations of total sulfide were quantitated to be 500 ± 140 and 1000 ± 300 μM (17 and 34 mg/L $H_2S_{(aq)}$).

Using a 10 mL Repeat-o-syringe pipet (Fisher Scientific) with a 20 gauge needle, 9 mL of the fortified buffer solution were slowly transferred to sterile 10 mL glass ampules (Wheaton, Millville, NJ). Each filled ampule was purged with a stream of nitrogen, then quickly sealed by an oxygen and natural gas flame. During this procedure, the fortified solutions were kept chilled in an ice bath.

Analysis of solutions

Duplicates of each buffer and $H_2S_{(aq)}$ concentrations were analyzed for EDB concentration, ^{14}C-activity, pH, E_h, and oxygen concentration. Sets of 14 ampules of each buffer and $H_2S_{(aq)}$ concentration were incubated at 25, 40, and 60°C ± 1.0°C in the dark. Ampules were analyzed at time intervals based upon EDB degradation rates determined in preliminary experiments.

At each time interval, ampules were allowed to come to room temperature before being opened. Two mL of the sample was withrawn for hexane extraction. One mL aliquots of both the hexane phase and the aqueous phase were transferred to separate scintillation vials containing 15 mL of liquid scintillation fluid and counted. The remaining 1.0 mL of hexane was transferred to a serum bottle for GC analysis of EDB. The GC analysis was performed on a Hewlett Packard 5890 GC with a model 3392 integrator/plotter and a 7671A autosampler and an ^{63}Ni electron capture detector. Grob technique injections were made onto a 25 m x 0.20 mm (film thickness, 0.33 μM, HP ULtra 1) capillary column temperature programed with an initial oven temperature of 40°C for 2 min, then increased to 90°C at 8°C/min, and finally increased at 30°C/min to 200°C. A carrier gas flow of Argon/5% methane was maintained at 1 mL/min. A column purge flow of 2 mL/min was activated at 1 min and a column make-up was 30 mL/min. In addition, 1.0 mL of the original

incubated solution was transferred to 15 mL of liquid scintillation fluid, shaken well, and then analyzed.

Total sulfide concentration was determined by the procedure of Cline (1969), as modified by Millero (1987). The method involves the formation of methylene blue from the sulfide and reagent solution of N,N-dimethyl-p-phenylene-diamine dihydrochloride and ferric chloride hexahydrate in acidifided solution. Color was developed in 30 min and absorbance was read at 670 nm with a Beckman DU8 spectrophotometer. Calibration solutions of concentrations ranging from 4 μM to 40 μM $H_2S_{(aq)}$ were prepared from a sodium sulfide stock solution for each analysis. Stock solutions of soduim sulfide were prepared by dissolving ca. 0.5 g of sodium sulfide crystals that were washed with deionized water and suctioned dry, in 100 mL of degassed deionized water. The stock solutions were standardized by the iodometric titration Standard Water snd Wastewater Method No. 427D (American Public Health Association, 1980, p 476). Concentrations of $H_2S_{(aq)}$ in experimental solutions were determined by comparison to a standard curve generated by least-squares regression analysis of the assay response vs. $H_2S_{(aq)}$ concentration.

EDB apparent first-order degradation rate constants were obtained by linear regression least squares fit of plots of ln percent EDB remaining vs time. The partitioning of ^{14}C-activity of the initial ^{14}C-EDB and its ^{14}C-products were plotted versus time.

Product Identification

Hexane extracts from incubated of EDB and $H_2S_{(aq)}$ in buffered solutions from kinetic experiments and the same buffer systems containing higher concentrations of reactants, i.e., 60 μM EDB and 1000 or 2000 μM $H_2S_{(aq)}$ were subjected to GC and GC/MS analyses. Standard solutions of 11 commercially available sulfur-containing compounds in hexane were chromatographed and subjected to mass spectrometry for comparison with products formed in experimental reaction solutions. Molecular formulae, structure and suppliers of these standard compounds are presented in Table 4.

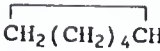
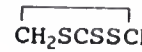
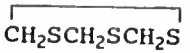
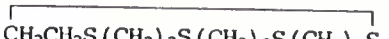
Ethylene Dibromide in Sulfate Groundwater

Groundwater from wells in the Florida Ambient Groundwater Network, which are known to have relatively moderate to high concentrations of sulfate, were obtained from officials of the South Florida Water Management District. Clean 1 L brown-glass screw-top bottles with Teflon-lined caps were filled in a manner which avoided excessive contact with the air and were kept on ice in an insulated cooler while being transported to the laboratory.

The pH, E_h and oxygen concentration of the samples were measured. Three mL aliquots were then extracted with 1.0 mL of hexane and the extracts analyzed by GC to determine if any detectable compounds were initially present.

Three sets of EDB-fortified solutions were prepared from each well water sample. Before fortification, one

Table 4. Commercially available sulfur-containing compounds used for product identification.

COMPOUND	ABBREVIATION	CHEMICAL FORMULA/ FORMULA WEIGHT	STRUCTURAL FORMULA	SUPPLIER/ PURITY
Ethanethiol CAS #75-08-1	ET	C_2H_6S 62.13	CH_3CH_2SH	Kodak (98%)
1-Butanethiol CAS #109-79-5	BT	$C_4H_{10}S$ 90.16	$CH_3(CH_2)_3SH$	Kodak (95+%)
1,2-Ethanedithiol CAS #540-63-6	EDT	$C_2H_6S_2$ 94.20	$HSCH_2CH_2SH$	Kodak (99.5%)
Cyclohexylthiol CAS #1569-69-3	CHT	$C_6H_{12}S$ 116.23		Aldrich NA
1,4-Dithiane CAS #505-29-3	DT	$C_4H_8S_2$ 120.24		Aldrich (97%)
Ethyl disulfide CAS #110-81-6	EDS	$C_4H_{10}S_2$ 122.25	$CH_3CH_2SSCH_2CH_3$	Aldrich (99%)
1,3-Dithiolane-2-thione CAS #822-38-8	DTT	$C_3H_4S_3$ 136.26		Aldrich (99%)
1,3,5-Trithiane CAS #291-21-4	TT	$C_3H_6S_3$ 138.27		Chem Service (99%)
Butyldisulfide CAS #629-45-8	BDS	$C_8H_{18}S_2$ 178.36	$CH_3(CH_2)_3SS(CH_2)_3CH_3$	Aldrich (98%)
1,4,7-Trithiacyclononane CAS #6573-11-1	TTCN	$C_6H_{12}S_3$ 180.36		Aldrich (98%)
1,4,7,10-Tetrathiacyclododecane CAS #25423-56-7	TTCD	$C_8H_{16}S_4$ 240.47		Aldrich (97%)

NA = Data not available

portion of each groundwater sample was autoclaved for 20 min (121°C, 15 psi), another portion was degassed by bubbling purified nitrogen gas through the water for approximately 2 hr, and a third portion of each water was left untreated.

Each portion of groundwater was fortified with a stock solution of unlabeled EDB to give 1.5 mg/L EDB and also with a stock solution of ^{14}C-EDB to give a concentration of approximately 3000 dpm/mL. Sets of 10 mL ampules were filled with about 8 mL of solution, the air space in the ampule purged with a stream of purified nitrogen, and then sealed by flame. The ampules were kept at 25°C in the dark and periodically analyzed by GC for EDB concentration and the appearance of extractable products. In addition, the partitioning of ^{14}C-EDB and ^{14}C-products after hexane extraction was determined using the method previously described. One mL aliquots of the solutions were also analyzed by liquid scintillation counting to check containment of the EDB during incubation.

Organic Synthesis of a Sulfur Product

The aliphatic cyclic sulfur-containing compound, 1,2,5,6-tetrathiacyclooctane is not commercially available, but its synthesis has been described by Goodrow et al. (1982). The procedure involves slowly reacting a dilute concentration of 1,2-ethanedithiol in chloroform with iodine and triethylamine (reagents concentration of approximately 10 μM, Goodrow et al., 1981).

RESULTS

Hydrolysis Reactions

Verification of Assay for Hydrolysis Products

Determination of EDB

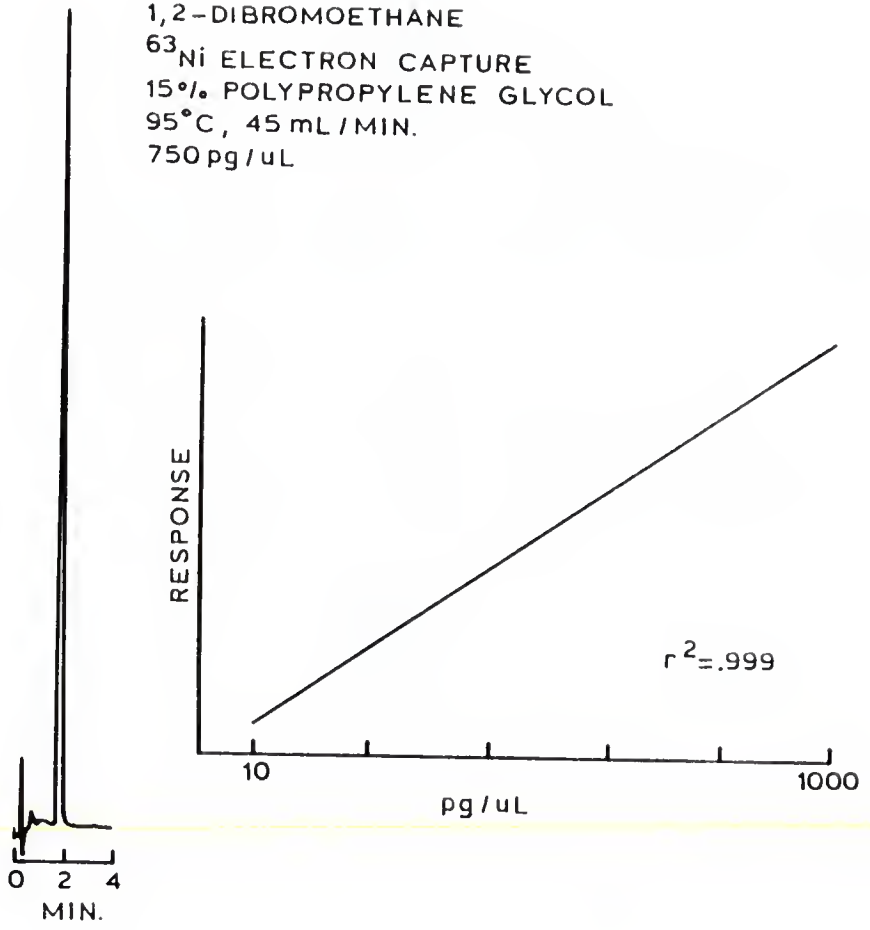
Using the conditions previously described, EDB in hexane extracts was quantitated using GC/EC by comparison of unknowns to calibration curves consisting of four or five standard solutions of EDB in hexane. The GC/EC response was linear and reproducible over a range of 10 to 1200 pg of EDB injected. Figure 2 shows a sample chromatogram and calibration curve. The efficiency of extraction of EDB from aqueous solutions into hexane was 95 to 100% over the concentrations range using both labeled and unlabeled EDB.

Determination of ethylene glycol

Procedures for quantitation of formaldehyde and ethylene glycol were validated using standard solutions para formaldehyde and 37% formaldehyde solution or ethylene glycol preparated in deionized water. In addition, ^{14}C-labeled analytes were employed (formaldehyde 150 mCi/mg, ethylene glycol 68 mCi/mg) for verifying the assay.

Since both analytes are very water-soluble and their 2-nitrophenylhydrazone derivatives formed in the analysis are extractable into hexane, partitioning of ^{14}C-activity was

Figure 2. Gas chromatogram, typical calibration curve and conditions for analysis of EDB.


1,2-DIBROMOETHANE
63Ni ELECTRON CAPTURE
15% POLYPROPYLENE GLYCOL
95°C, 45 mL/MIN.
750 pg/uL
RESPONSE
r2=.999
10
1000
pg/uL
0 2 4
MIN.

used to test efficiency of the assay. Because ^{14}C-activity can be measured at extremely low concentrations of labeled material, a very sensitive assessment of efficiency was used to test efficiency of the assay. Because ^{14}C-activity can be measured at extremely low concentrations of labeled material, a very sensitive assessment of efficiency was possible.

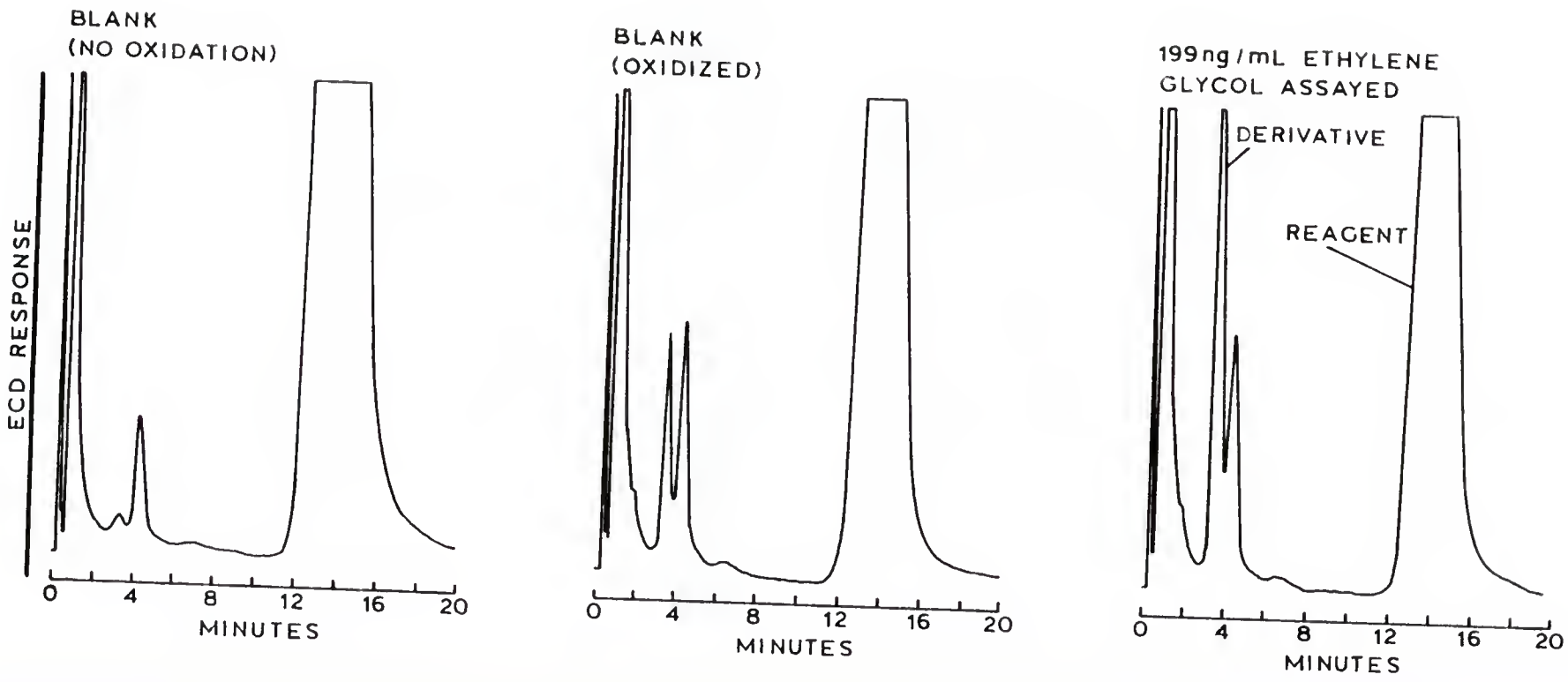
After derivatizing six solutions ranging from 17 to 689 µg/L of ^{14}C-formaldhyde, 86 to 98% was recovered in the hexane extract. Performing the ethylene glycol procedure on four solutions containing 288 to 2878 µg/L of the compound resulted in the recovery of 89% to 100% of the labeled compound in the hexane extract.

Calibration curves for ethylene glycol were typically constructed over a concentration range of 60 to 1500 µg/L. Resulting correlations of determination (r^2) of linear regression least squares analyses were larger than 0.99. Figure 3 shows chromatograms of blanks and results of the assay of ethylene glycol. Background concentrations of formaldehyde between 10 and 50 µg/L are common and must be used to correct the results derived from the analysis of unknown samples.

Kinetics: Temperature and pH Dependence

The disappearance of EDB in fortified groundwaters and deionized water was followed over 22 days for incubation at 40, 50 and 60°C, 17 days for incubation at 70°C, and 6 days

Figure 3. Gas chromatograms after the 2-nitrophenylhydrazine derivatization assay of unoxidized blank, oxidized blank and ethylene glycol (199 μg/L).


BLANK
(NO OXIDATION)
ECD RESPONSE
0
4
8
12
16
20
MINUTES
BLANK
(OXIDIZED)
0
4
8
12
16
20
MINUTES
199ng/mL ETHYLENE
GLYCOL ASSAYED
DERIVATIVE
REAGENT
0
4
8
12
16
20
MINUTES

for incubations at 80°C. These changes correspond to decreases greater than 90% of initial concentrations of EDB in the 80°C incubations and approximately 20% decreases of EDB at 40°C. At intermediate temperatures, the degree of degradation fell within this range. Linearity in plots of the log % EDB concentration remaining vs. time demonstrated apparent first-order degradation rates. The least-squares regression coefficients of determination (r^2) were greater than 0.99 at higher temperatures and greater than 0.90 at lower temperatures. The rate data from the Polk County groundwater sample initially fortified to a concentration of 100 μg/L EDB are shown in Figure 4. If EDB degradation in solution was an acid- or base catalyzed reaction, non-linear semi-logarithmic plots of observed rate constants vs pH should result. Figure 5 shows the observed rate constants for the degradation of EDB in the groundwaters and deionized water as a function of pH while incubated at 63°C. Table 5 shows the observed rate constants for the laboratory EDB-fortified waters. The rate constants differ only slightly between the waters and indicate neither acid- nor base-catalyzed degradation. The larger rate constants observed at pH 5 and pH 8 as compared to those of pH 7 are not consistent with a pH dependent degradation process. The boric acid buffer at pH 7.8 to 8.3 may have participated in EDB degradation, thereby increasing the observed rate constant. Buffers can catalyze hydrolysis even when present at low mM

Figure 4. Disappearence of EDB at elevated temperatures in Polk County groundwater initially containing 100 ug/L EDB.

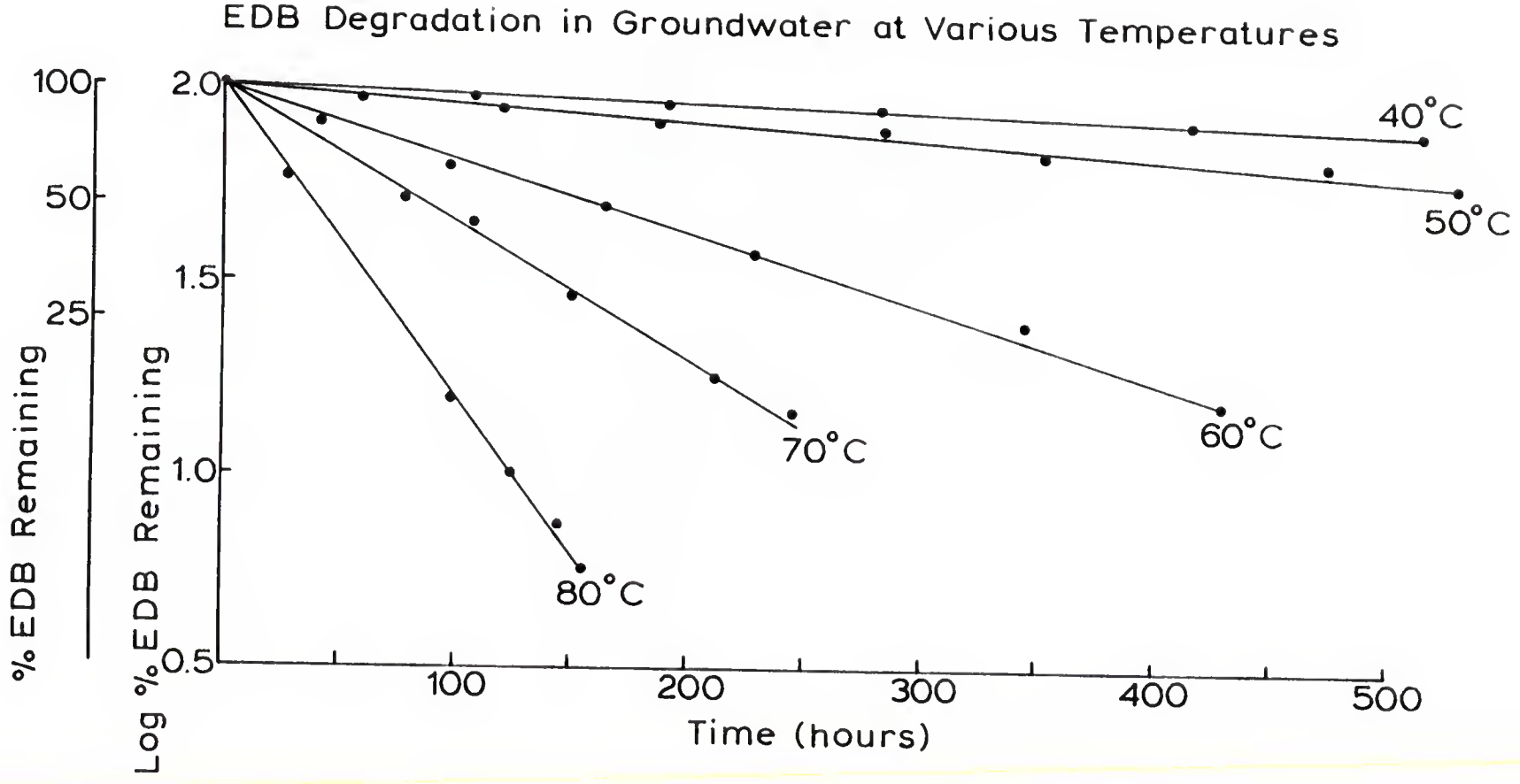

EDB Degradation in Groundwater at Various Temperatures
100
50
25
% EDB Remaining
2.0
1.5
1.0
0.5
Log % EDB Remaining
40°C
50°C
60°C
70°C
80°C
100
200
300
400
500
Time (hours)

Figure 5. Observed EDB degradation rate constants as a function of pH in buffered solutions (0.005 M 63°C). Buffers are identified in Table 5.

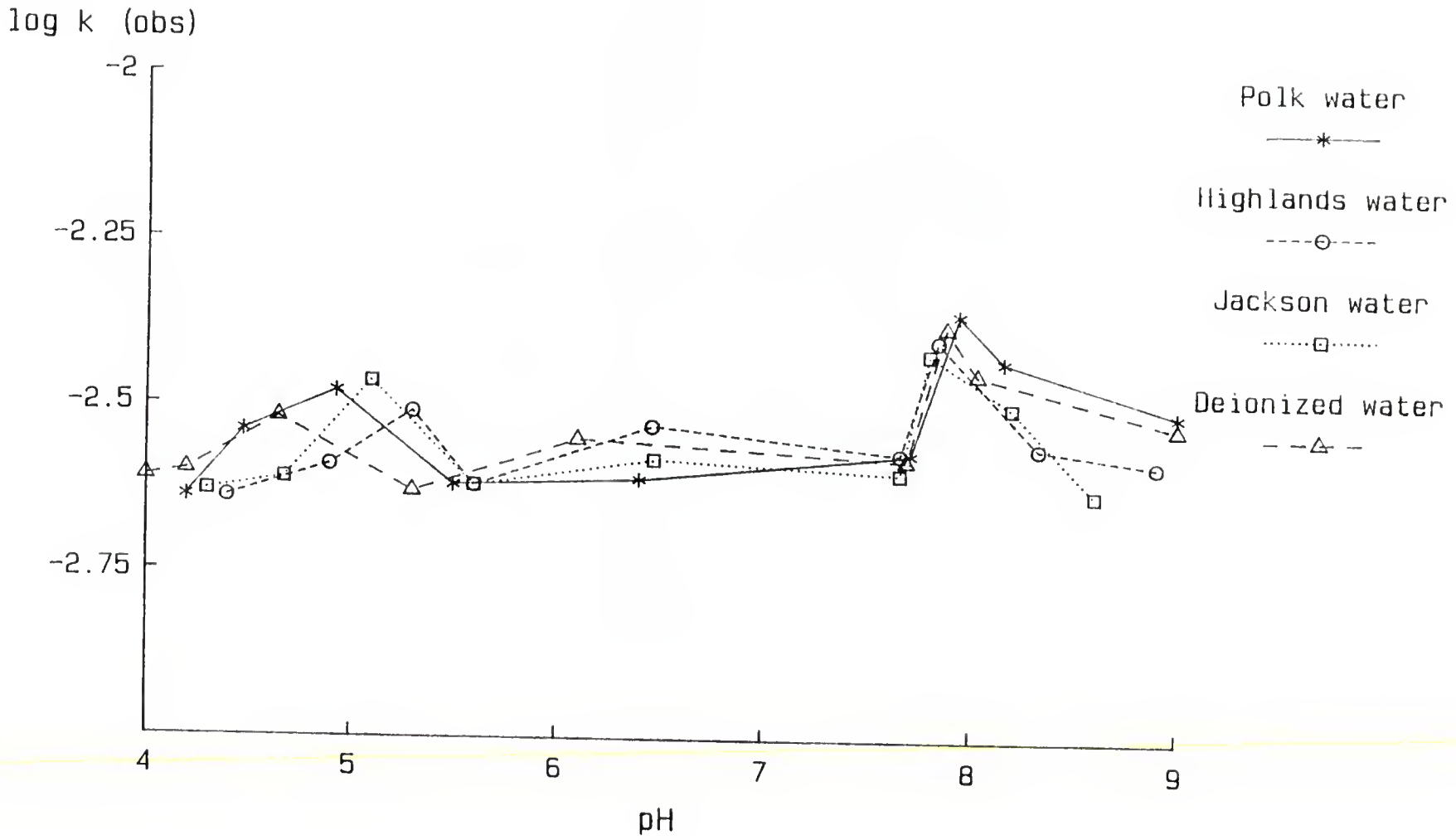

log k (obs)
-2
-2.25
-2.5
-2.75
4
5
6
7
8
9
pH
Polk water
Highlands water
Jackson water
Deionized water

Table 5. The effect of pH on the EDB degradation rate at 63°C and initial EDB concentration of 100 μg/L.

pH	Buffer[a]	10^3k (hr^{-1})[b] ($t_{1/2}$, hr)				$10^3k \pm SD$
		Polk	Highlands	Jackson	Deion.	
4.0-4.4	a	2.31 (301)	2.31 (302)	2.34 (298)	2.48 (280)	2.36±0.13
4.0-4.9	b	2.94 (236)	2.61 (266)	2.45 (283)	2.54 (273)	2.63±0.29
4.7-5.3	b	2.84 (208)	2.56 (225)	3.46 (200)	3.10 (225)	3.23±0.45
5.3-5.6	a	2.41 (288)	2.44 (288)	3.47 (287)	3.10 (295)	2.40±0.10
6.1-6.5	c	2.45 (283)	2.98 (233)	2.64 (263)	2.83 (285)	2.73±0.23
7.6-7.7	d	2.68 (259)	2.68 (259)	2.52 (275)	2.63 (263)	2.95±0.62
7.8-7.9	e	4.39 (158)	3.96 (175)	3.83 (181)	4.17 (166)	4.17±0.38
8.0-8.3	f	3.76 (266)	2.81 (247)	3.56 (195)	3.62 (191)	3.44±0.44
8.6-9.0	g	3.07 (224)	2.61 (267)	2.41 (288)	2.95 (235)	2.95±0.48

[a]0.005 M buffers: a = borax/succinic acid
b = potassium phthalate
c = borax/succinic acid
d = borax/phosphate
e,f = boric acid
g = carbonate

[a]Apparent First-order rate constant for degradation of EDB
Average of replicate kinetic runs with 6 data points each
Half-life, $t_{1/2} = 0.693/k$

[c]All samples in pH range, n=8

concentrations (Perdue and Wolfe, 1983). The observed first-order EDB degradation rates were used to construct Arrhenius plots like the one shown in Figure 6. The data shown are from EDB degradation in Polk County groundwater. The least-squares regression coefficients of determination (r^2) for these plots ranged from 0.97 to 0.99 for all the EDB-fortified waters tested. The resulting Arrhenius kinetic parameters were used to make estimates of EDB degradation rates at ambient groundwater temperatures. In Table 6, the extrapolated kinetic parameters derived from these data are shown for 22°C, a common groundwater temperature in Florida. The kinetic parameters do not significantly vary with the source of the water or with the initial concentration of EDB. At 22°C in the waters tested, the predicted half-life ($t_{1/2}$) ranges from 259 to 772 days. The activation energy ranges from 19.1 to 24.3 kcal mol^{-1}. The variation among the data is fairly large, with standard deviations generally ranging from 12 to 20% of their respective means, except for J10 where the standard deviation is 58% of the mean. Since the extrapolated values for rate constants at ambient temperatures are derived from relatively few data points a fair degree of error is expected. Sources of error in rate constant may arise from small deviations in temperature of experimental incubations and random error (Harris, 1982; Maybey and Mill, 1978).

Figure 6. Arrhenius plot illustrating hydrolysis of EDB in Polk County groundwater initially containing 100 ug/L EDB.

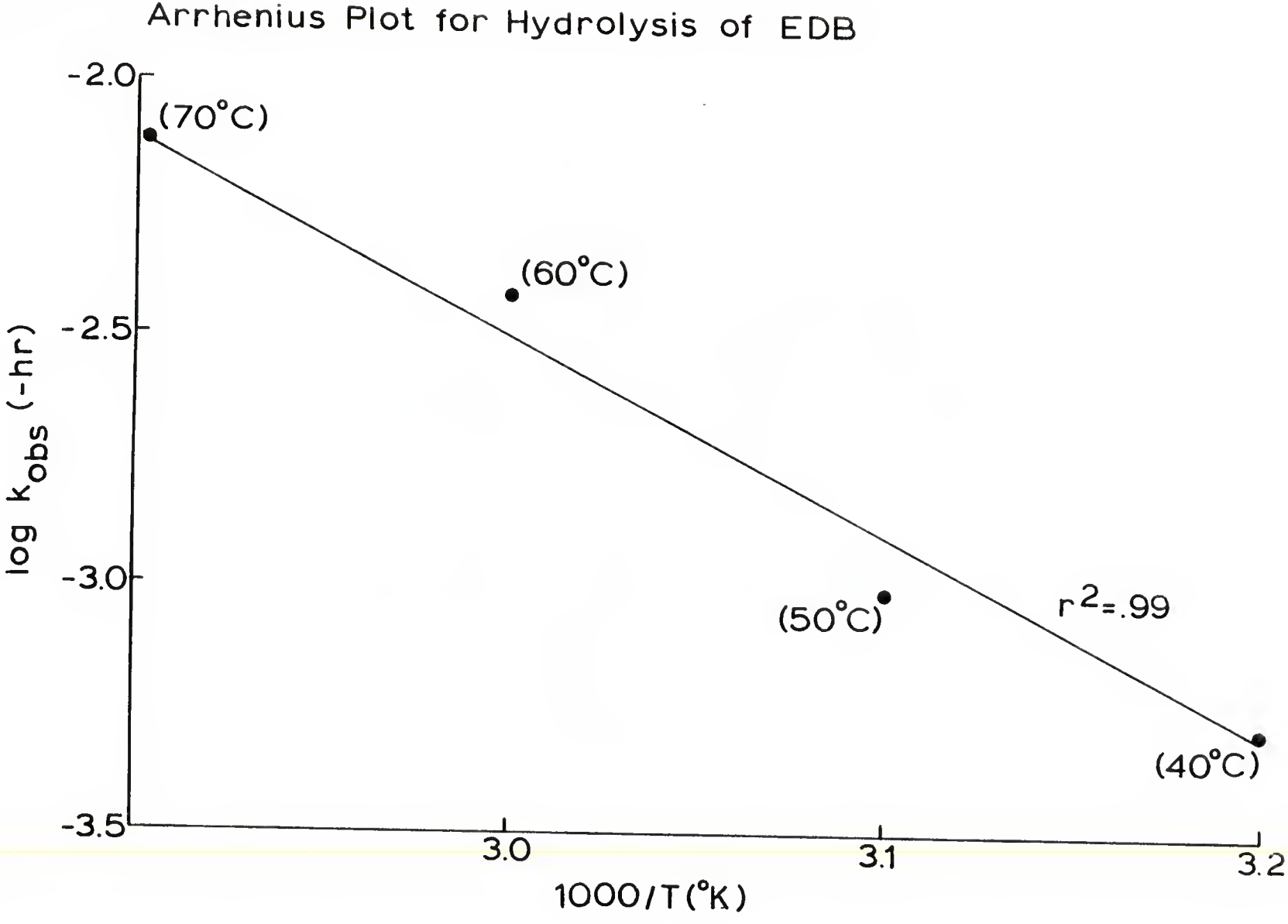

Arrhenius Plot for Hydrolysis of EDB
-2.0
-2.5
-3.0
-3.5
log k_{obs} (-hr)
(70°C)
(60°C)
(50°C)
(40°C)
r^2=.99
3.0
3.1
3.2
1000/T (°K)

Table 6. EDB degradation kinetic parameters in solutions studied as predicted by Arrhenius kinetics plots. Extrapolated values for 22°C are shown.

Sample[a]	10^3k[b] 22°C, (d^{-1})	$t_{½}$[c], 22°C, (d^{-1})	Ea[d] (kcal mol^{-1})	Log A[e] (d^{-1})
P10	2.76 ±0.41	259 ± 35	19.6 ±0.2	12.2 ± 0.5
P100	1.61 ±0.19	434 ± 47	20.9 ±0.4	12.7 ± 0.2
H10	0.64 ±0.38	772 ± 94	24.2 ±0.8	14.9 ± 0.6
H100	2.33 ±0.57	309 ± 67	19.1 ±0.6	11.6 ± 0.4
J10	1.52 ±0.65	547 ±317	21.1 ±2.2	12.1 ± 0.3
J100	1.93 ±0.36	369 ± 69	19.7 ±0.6	11.9 ± 0.4
DW10	2.15 ±0.25	326 ± 38	19.6 ±0.6	11.8 ± 1.7
DW100	2.15 ±0.25	326 ± 38	19.6 ±0.6	11.7 ± 1.7

[a] P=Polk county groundwater
H=Highlands county groundwater
J=Jackson county groundwater
DW = deionized water
10 = 10 μg/L, concentration of EDB fortification
100= 100 μg/L, concentration of EDB fortification

[b] Mean of four replicate runs, except for two replicates of DW samples;
mean of pseudo-first order rate constant ± standard deviation, except DW is mean ± range of replicates

[c] Mean ± standard deviation of half-lives of EDB in days, except DW is mean ± range of replicates

[d] Mean ± standard deviation of Arrhenius activation energy, except DW is mean ± range of replicates

[e] Mean ± standard deviation of frequency factor, except DW is the mean ± range of replicates

Product Identification

In experiments using ^{14}C-EDB conducted at elevated temperatures, the decline in ^{14}C-activity in the hexane phase, determined after extraction of incubated solutions, closely paralleled the degradation of EDB, as quantitated by GC/EC (Figure 7). These observations are highly reproducible, as shown in the results from simultaneous quantitation and partitioning of ^{14}C-EDB and ^{14}C-products during incubation at 80°C (Table 7). At the completion of the experiment, the total ^{14}C-activity in the solutions ranged from 92 to 99 percent of the initial activity. This finding indicates that no EDB or product was lost during the experiment and that no products volatilized. The next experiments employed solutions containing higher initial concentrations of EDB (ca. 3.5 mg/L) than in the prior experiments for the purpose of quantitating the water-soluble products formed, i.e., bromide ion and ethylene glycol (Figures 8a, 8b and 8c). Total ^{14}C-activity was also quantitated along with the hexane/aqueous partitioning of ^{14}C-activity during incubation of the EDB-fortified groundwater and deionized water. During the 17 day incubations conducted at 73°C, the amounts of bromide ion and ethylene glycol together accounted for 60 to 70% of the initial EDB present. The extent of EDB degradation in these incubation was approximately 8 or 9 half-lives. As in previous experiments, the partitioning of ^{14}C-activity into

Figure 7. Partitioning of ^{14}C-activity (^{14}C-EDB and ^{14}C-products by liquid scintillation counting) during EDB degradation at 80°C.

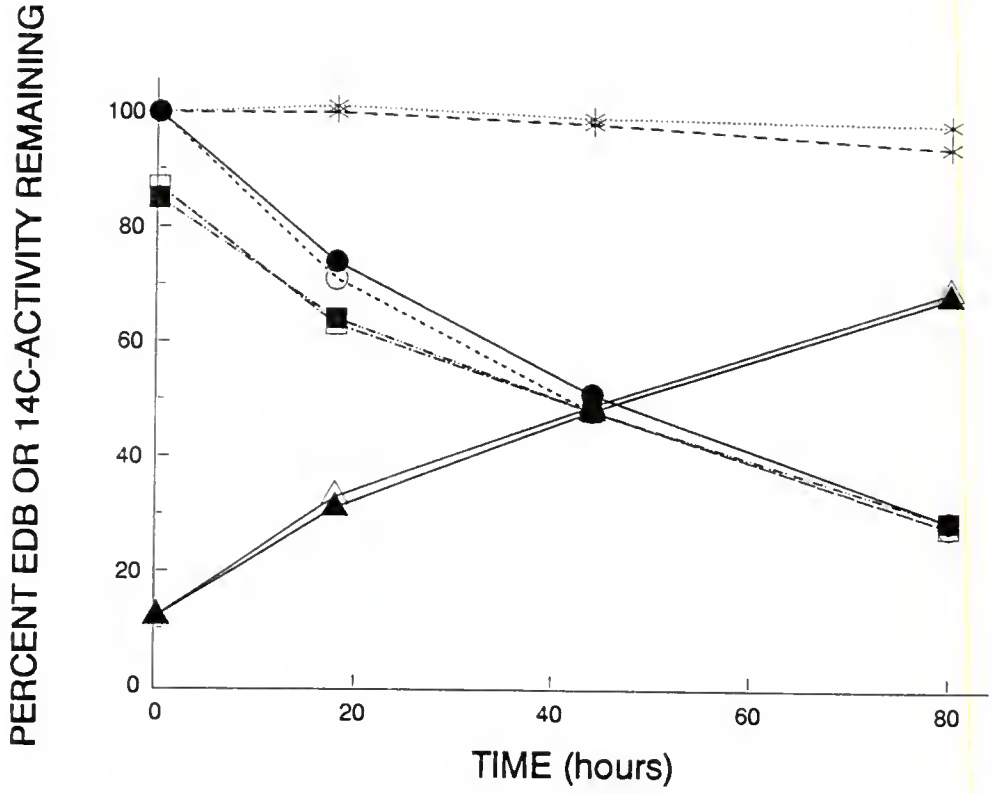

PERCENT EDB OR 14C-ACTIVITY REMAINING
100
80
60
40
20
0
0
20
40
60
80
TIME (hours)
EDB CONCENTRATION 1
EDB CONCENTRATION 2
TOTAL 14C-ACTIVITY 1
TOTAL 14C-ACTIVITY 2
HEXANE 14C-ACTIVITY 1
HEXANE 14C-ACTIVITY 2
AQUEOUS 14C-AQUEOUS1
AQUEOUS 14C-AQUEOUS 2

Table 7. Simultaneous quantitation of EDB (GC/EC) and partitioning of ^{14}C-activity (^{14}C-EDB and ^{14}C-products by liquid scintillation counting) in dilute solution during degradation at 80°C.

Time/Sample[a]	[EDB] (μg/L)	^{14}C in Hexane (dpm/mL)	^{14}C in Aqueous (dpm/mL)	^{14}C Total (dpm/mL)
0 Hour				
A1	56.4	150774(85)	19815(11)	177950
A2	59.8	150740(84)	23425(13)	178550
B1	28.9	77950(86)	10535(12)	90700
B2	29.1	78221(88)	10495(12)	88970
18 Hour				
A1	41.5(74)[b]	114456(64)	55464(31)	178230(100)
A2	41.9(70)	115752(64)	55860(31)	181680(102)
B1	21.0(73)	53918(60)	30415(34)	89940(99)
B2	20.1(69)	58349(65)	28965(32)	89270(100)
44 Hour				
A1	29.5(52)	83216(48)	83315(48)	175190(98)
A2	29.5(49)	82673(47)	84900(48)	176560(99)
B1	14.5(50)	42196(48)	43405(49)	88590(98)
B2	13.8(47)	41330(47)	42765(49)	87360(96)
80 Hour				
A1	16.3(29)	51285(30)	117290(68)	172890(97)
A2	16.5(28)	48424(28)	117025(67)	175880(99)
B1	8.1(28)	23402(27)	59705(69)	87110(96)
B2	7.8(26)	23942(29)	56695(69)	82050(92)

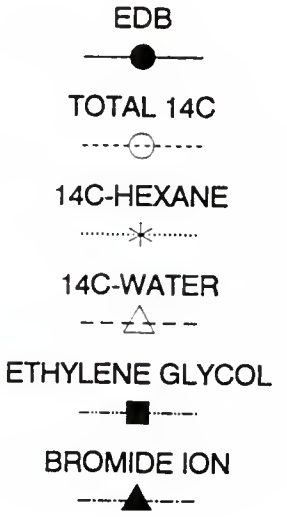
EDB Degradation Kinetic Data (first-order plot)

	Regression Equation		Apparent rate constant (hr^{-1})
A1	y= -0.00667x + 2.00	r^2= -.9990	1.54×10^{-2}
A2	y= -0.00689x + 1.99	r^2= -.9989	1.59×10^{-2}
B1	y= -0.00679x + 1.99	r^2= -.9994	1.56×10^{-2}
B2	y= -0.00699x + 1.99	r^2= -.9983	1.61×10^{-2}

[a]10 mL aliquots of 100 mL samples are taken for each analysis interval; samples 1 and 2 are duplicates
[b]Percent of 0 hour measurement in parenthesis

Figure 8. Partitioning of ^{14}C-EDB and ^{14}C-products and quantitation of EDB, ethylene glycol, and bromide during incubation in Florida groundwater and deionized water. Each point is an average of replicate measurements.

8a. EDB-fortifed Polk and Highlands groundwater incubated at 73°C.



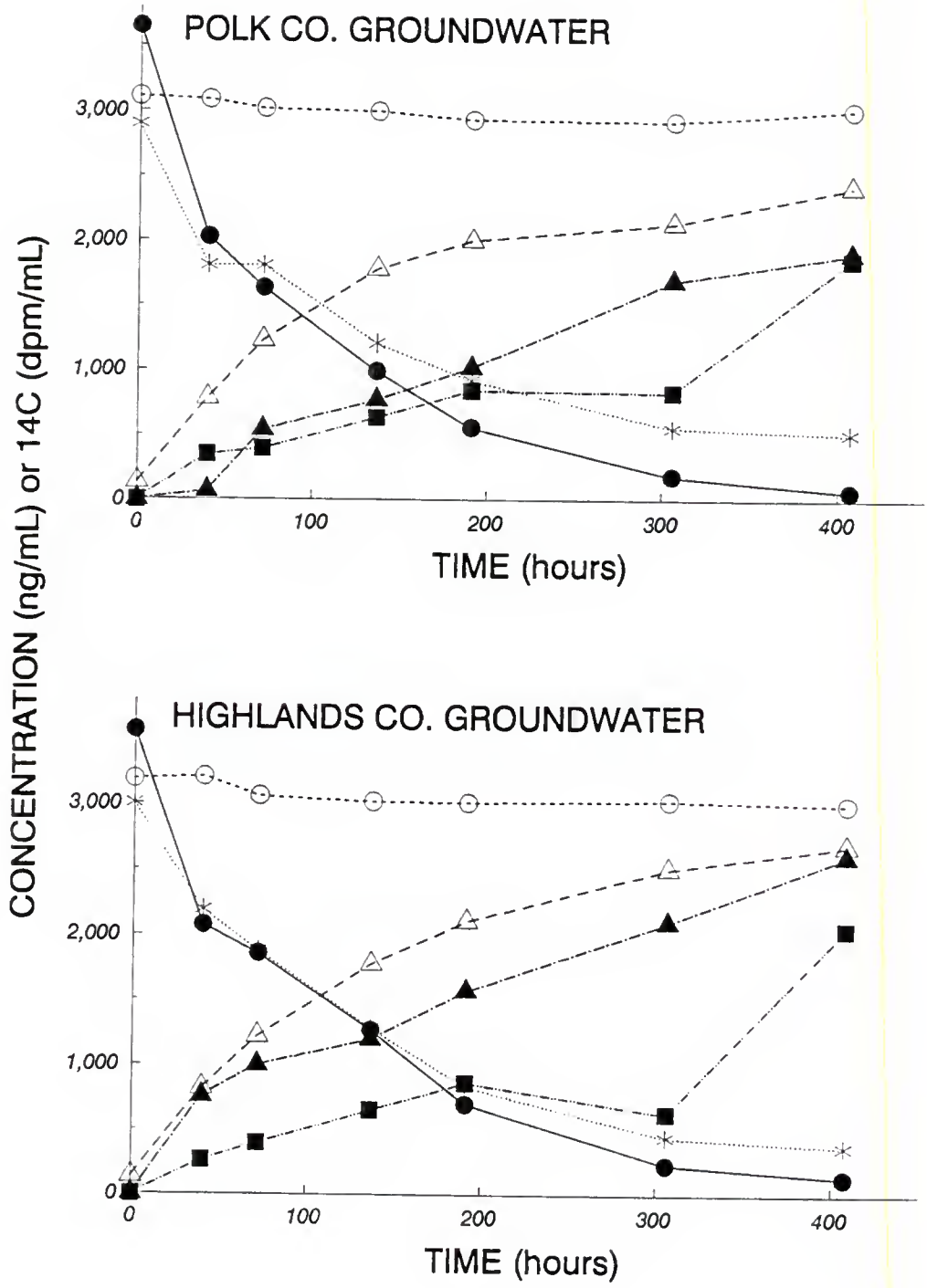
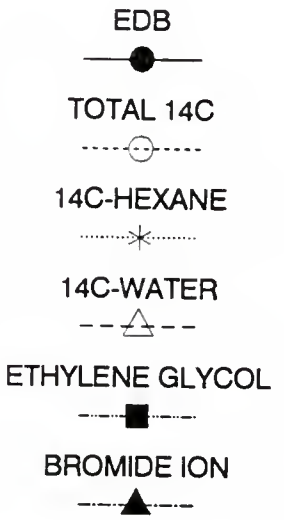

POLK CO. GROUNDWATER
HIGHLANDS CO. GROUNDWATER
CONCENTRATION (ng/mL) or 14C (dpm/mL)
TIME (hours)
TIME (hours)
0
1,000
2,000
3,000
0
100
200
300
400

Figure 8 continued.

8b. EDB-fortified Jackson groundwater and deionized water incubated at 73°C.



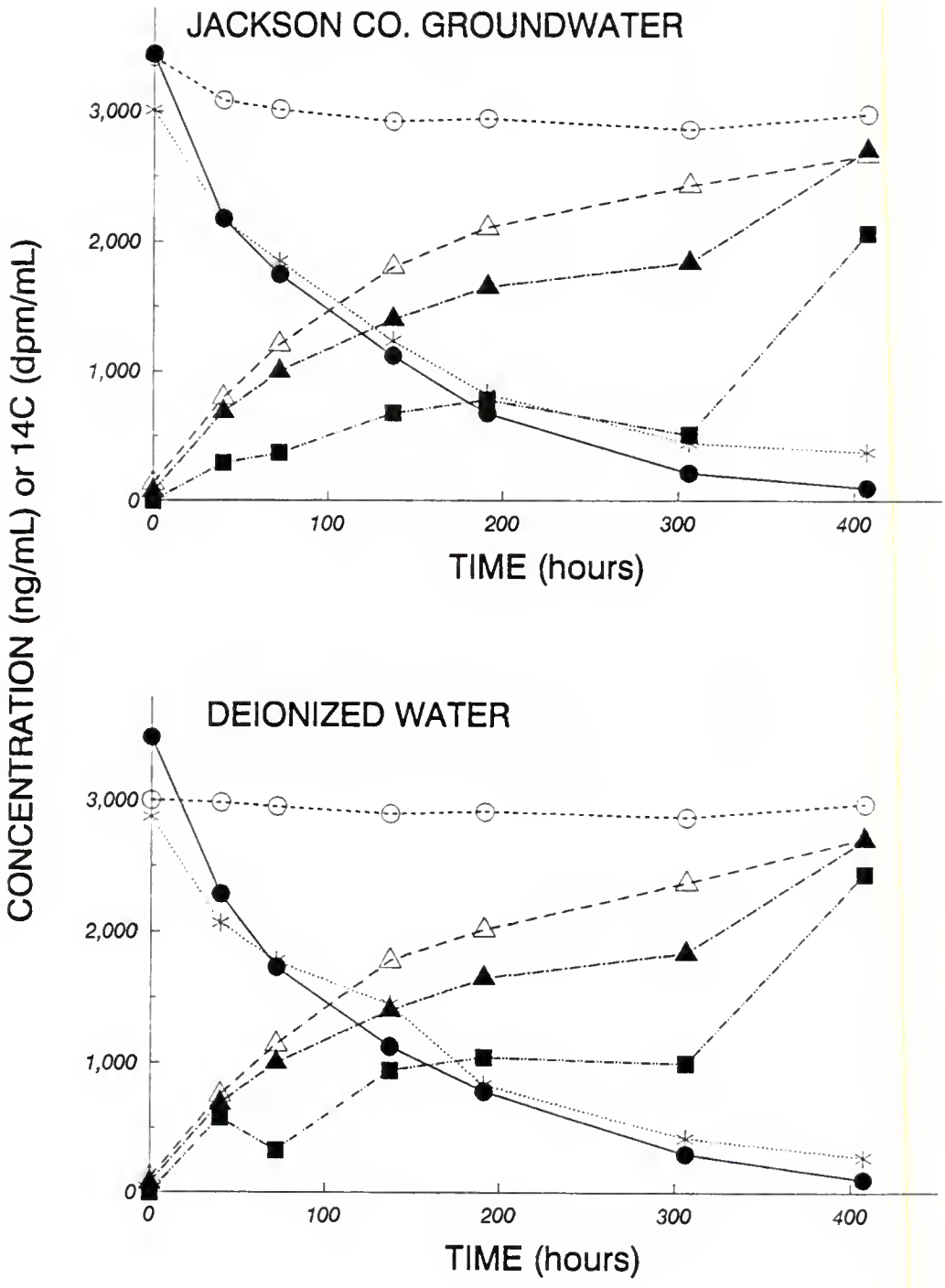
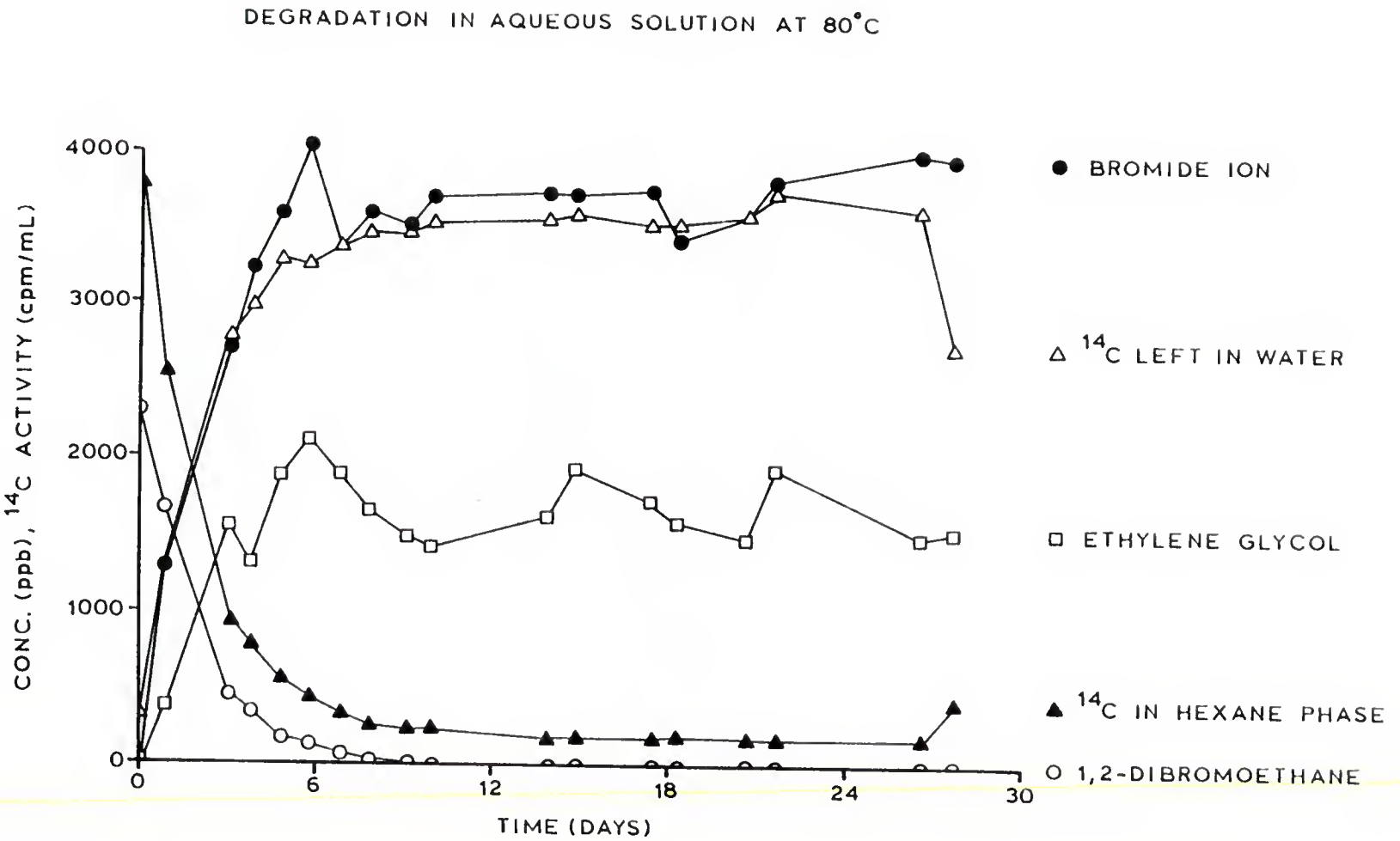

JACKSON CO. GROUNDWATER
DEIONIZED WATER
CONCENTRATION (ng/mL) or 14C (dpm/mL)
3,000
2,000
1,000
0
0
100
200
300
400
TIME (hours)

Figure 8 continued.

8c. EDB-fortified deionized water incubated at 80°C.


DEGRADATION IN AQUEOUS SOLUTION AT 80°C
CONC. (ppb), ^{14}C ACTIVITY (cpm/mL)
4000
3000
2000
1000
0
0
6
12
18
24
30
TIME (DAYS)
BROMIDE ION
^{14}C LEFT IN WATER
ETHYLENE GLYCOL
^{14}C IN HEXANE PHASE
1,2-DIBROMOETHANE

the aqueous phase after extraction closely paralleled the decline in EDB concentration. Similar patterns of transformation of EDB were seen in all three fortified groundwaters and in deionized water. Figure 8c shows the pattern of EDB transformation during an incubation at a higher temperature (80°C) which was observed for a longer time period (27 days) in deionized water. In this experiment, the extent of conversion of EDB to bromide ion and ethylene glycol ranged between 70 and 90%. Further examination of extractions of aliquots from solutions in this experiment did not reveal either additional brominated products of EDB or intermediates in the conversion of EDB to bromide ion and ethylene glycol. Purge-and-trap GC/MS analyses of aqueous aliquots detected only trace quantities of 3- and 5-member hydrocarbon compounds and remaining EDB.

Vinyl bromide could be formed from EDB if EDB were reductively dehalogenated. This compound was found by Vogel and Reinhard (1986) in pentane extracts of incubated aqueous EDB solutions. Although it could not be detected in any of the solutions or extracted by GC/EC or by purge-and-trap GC/MS in this study. Under the GC/EC conditions used in this study, the detection limit is at least 1000 times less sensitive for vinyl bromide compared to EDB. If vinyl bromide were formed in the present study, however, ^{14}C-vinyl bromide would have partitioned into hexaxe upon extraction. There was no indication that this occurred. Injections on

GC/EC were made of the hexane phase and aqueous phases of three replicate extractions of deionized water containing 1000 mg/L vinyl bromide. Vinyl bromide was only found in the hexane phase and not in the aqueous phase.

Extraction of 2-bromoethanol from dilute aqueous solutions was 30 to 40% efficient. Furthermore, GC/EC detection of this compound was also much less sensitive than EDB; at least 100 times less than EDB.

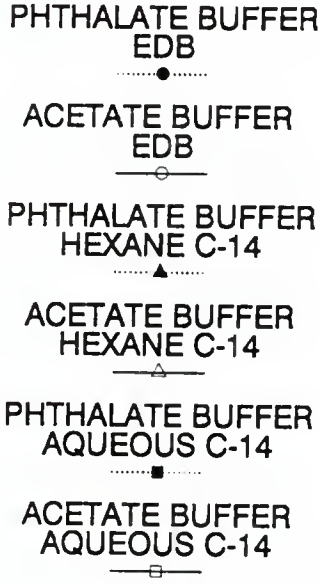
Reactions with Aqueous Hydrogen Sulfide

Kinetics: Temperature and pH Dependence

During incubations at 25, 40 and 60°C, the disappearance of EDB from pH and redox buffered solutions was examined along with the partitioning of ^{14}C-EDB and ^{14}C-products after hexane extraction (Figures 9a, 9b and 9c). These control solutions, which did not contain $H_2S_{(aq)}$, were studied over 237 days at 25°C, 194 days at 40°C and for 105 days at 60°C. At 25°C, neither the concentration of EDB nor the partitioning of the ^{14}C-EDB between the hexane and aqueous phases changed noticeably at pH 5, 7, or 9. At 40 and 60°C, the disappearance of EDB was paralleled by the changes in partitioning of the ^{14}C-activity. This transformation of EDB to water-soluble products in pH and redox buffered solutions was also observed in the groundwater and deionized water experiments discussed previously.

Figure 9. Degradation of EDB and partitioning of ^{14}C-products in buffered reducing solutions as a function of pH and temperature. Each point is the average of duplicate analyses.

9a. Solutions buffered at pH 5.



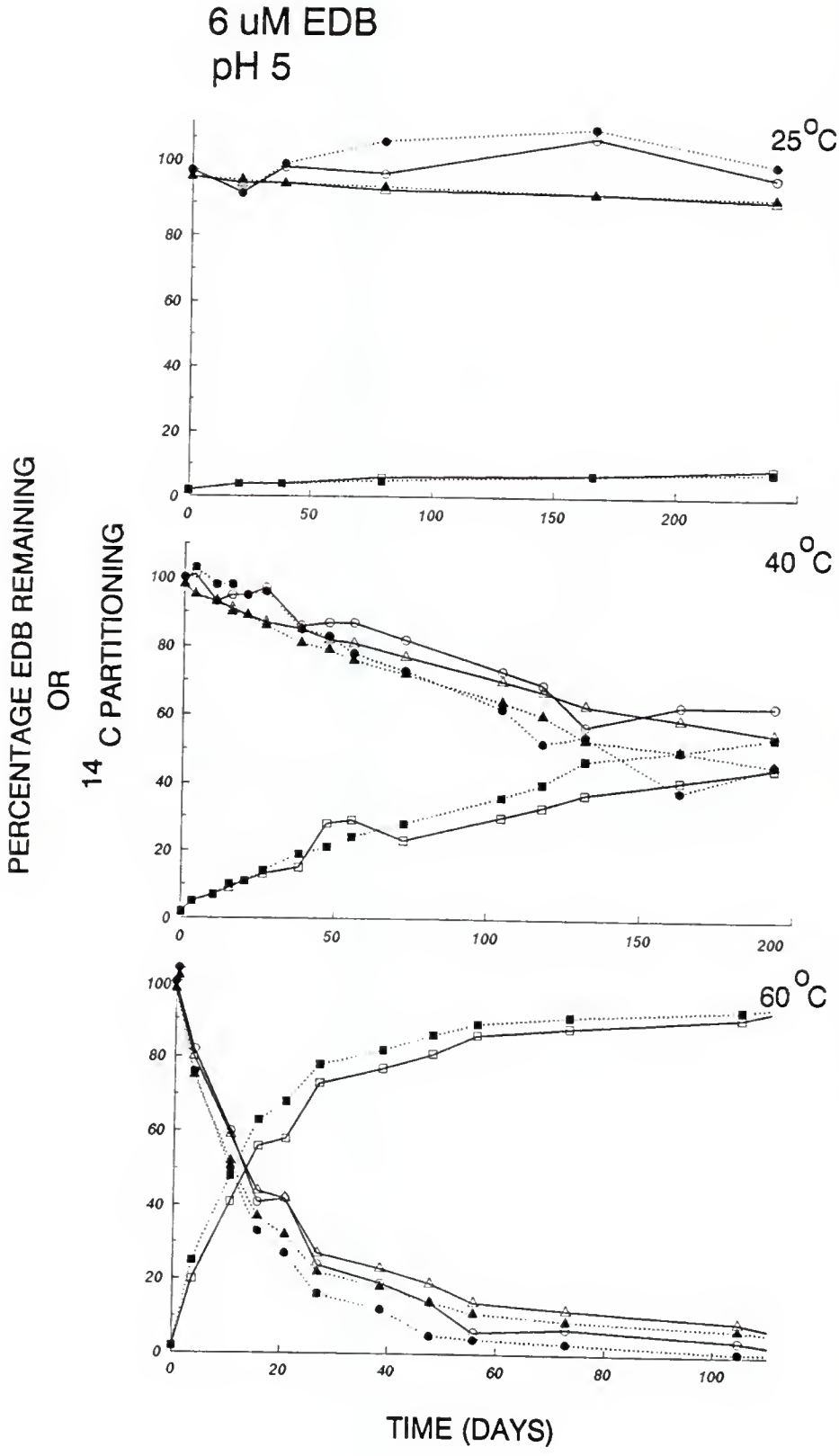
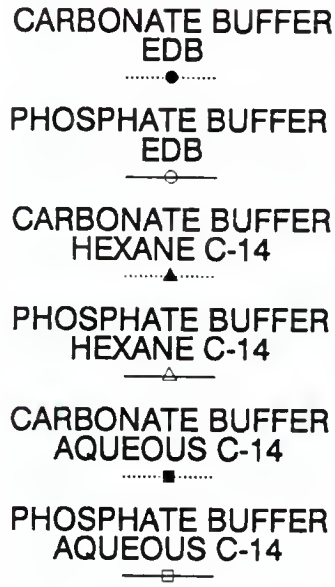

6 uM EDB
pH 5
25°C
40°C
60°C
PERCENTAGE EDB REMAINING
OR
14C PARTITIONING
TIME (DAYS)

Figure 9 continued.

9b. Solutions buffered at pH 7.



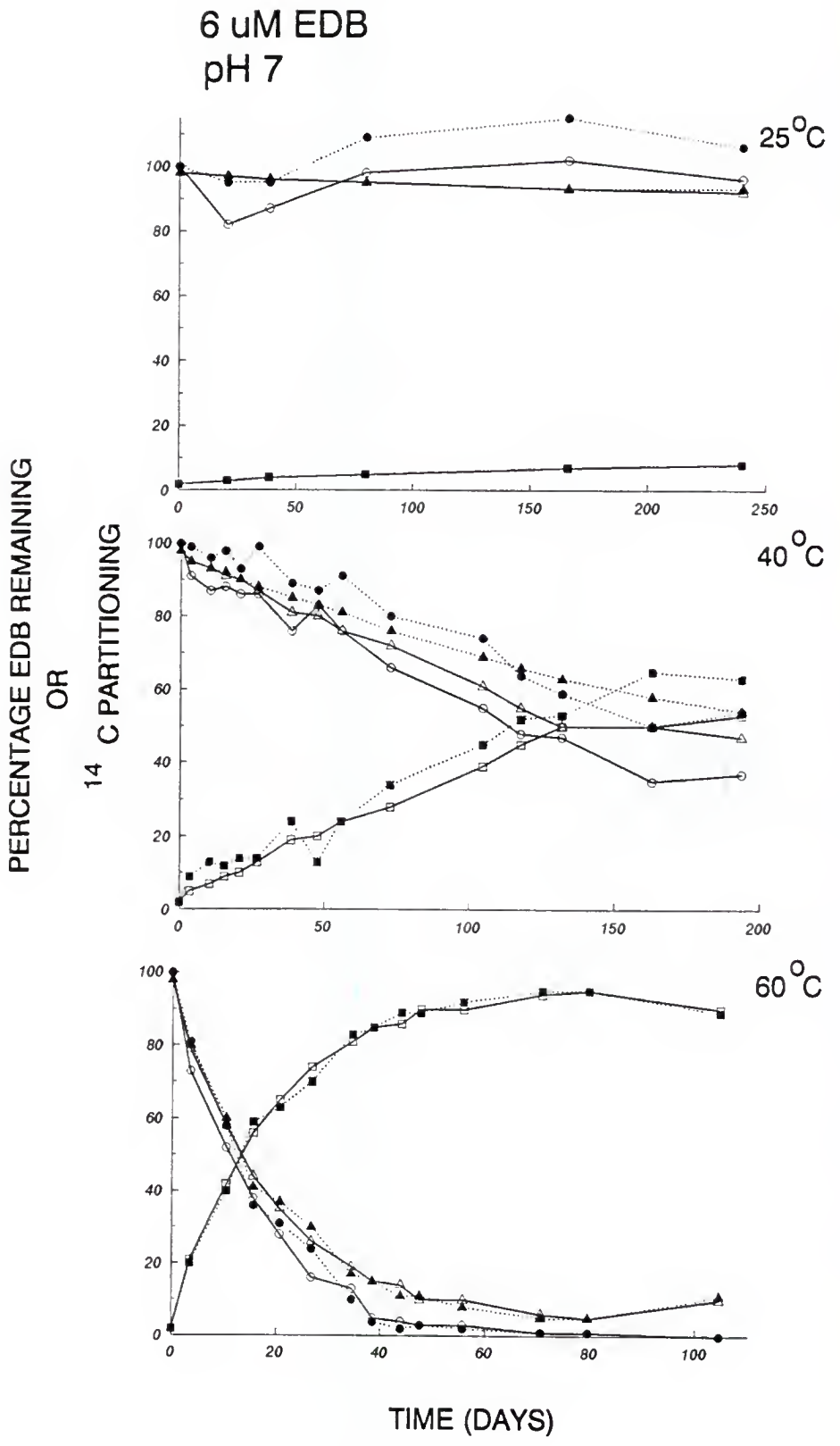
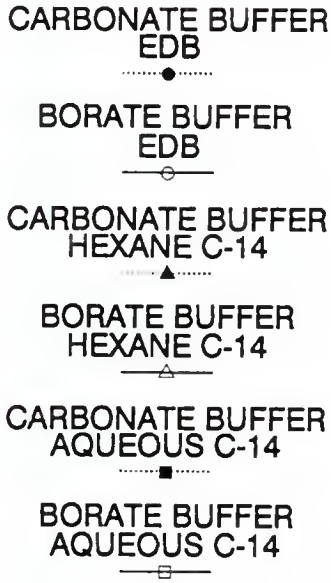
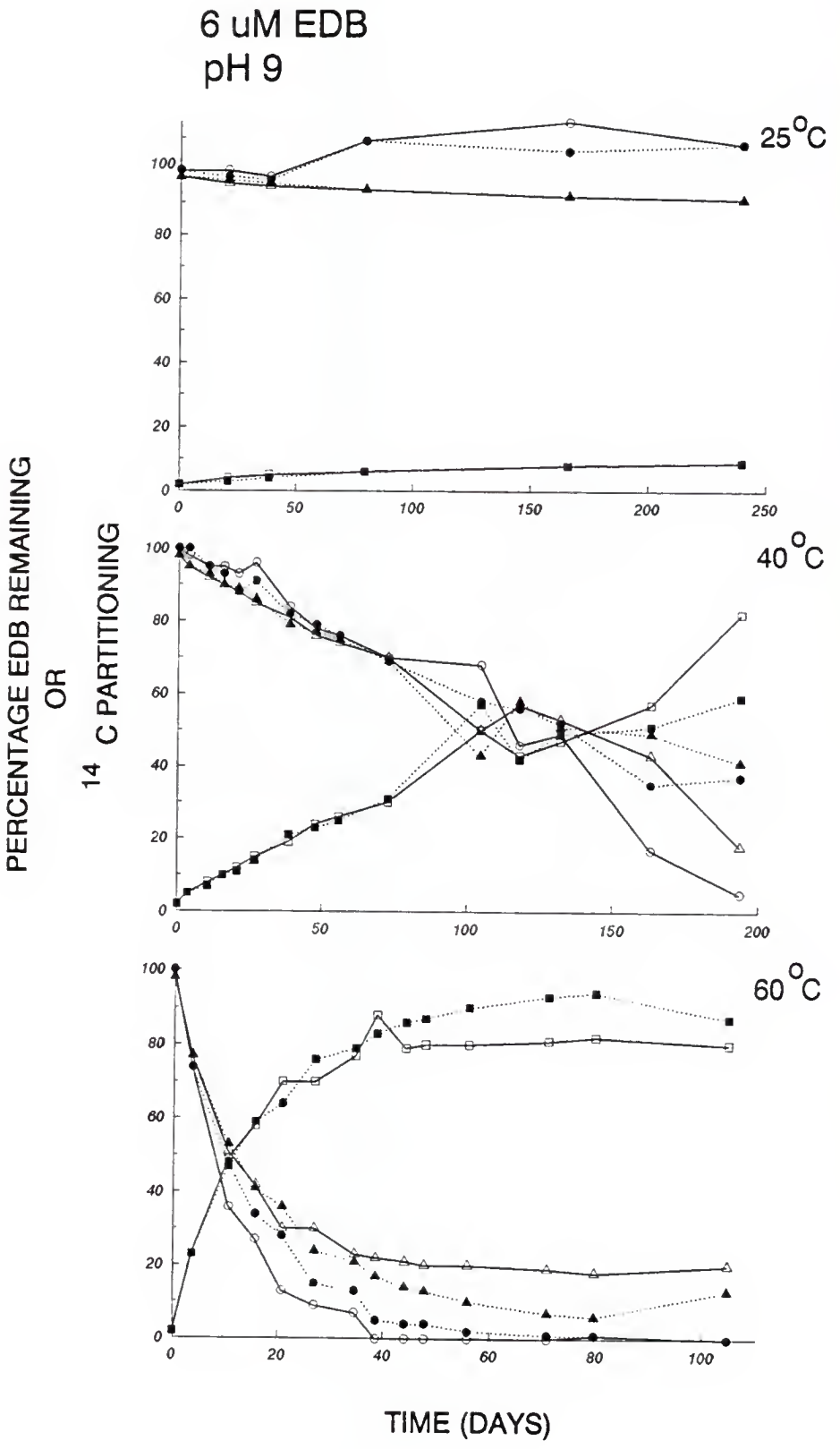

6 uM EDB
pH 7
25°C
40°C
60°C
PERCENTAGE EDB REMAINING
OR
14C PARTITIONING
TIME (DAYS)

Figure 9 continued.

9c. Solutions buffered at pH 9.



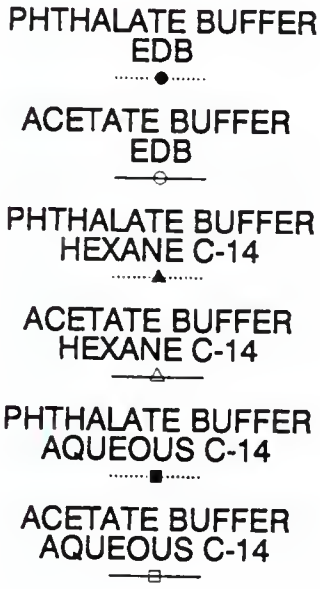

6 uM EDB
pH 9
25°C
40°C
60°C
PERCENTAGE EDB REMAINING
OR
14 C PARTITIONING
TIME (DAYS)

The type of pH buffer system (phthalate versus acetate; carbonate versus phosphate) had little effect on the results. One exception to this trend was the pH 9 buffer system (borate versus carbonate). A rate enhancement-effect of borate (but not carbonate) on the degradation of EDB was also observed in the previous study of the effect of pH on EDB hydrolysis.

Where EDB was monitored through more than one half-life, its degradation followed apparent first-order decay. EDB, initially present in a concentration of 6 μM, was no longer detectable at 100 days in each of the three pH buffering systems held at 60°C. In solutions also containing ca. 500 or 1000 μM $H_2S_{(aq)}$, very different patterns of EDB's transformation emerged (Figures 10a - 10f) and are described below. These solutions contained the same composition of pH and redox buffers as the controls described above. These solutions were held at 25°C and monitored for 254 days at pH 5 and 7 and for 150 days at pH 9. At 40°C, solutions of pH 5 and 7 were monitored for 93 days and for 82 days at pH 9. At 60°C, the solutions at pH 5 and 7 were watched for 62 days and for 19 days at pH 9. At the start of the incubation of these solutions, the dissolved oxygen was less than 0.07 ppm. The initial redox potentials for the solutions buffered at pH 5, 7 and 9 ranged from -240 to -258 mV, -238 to -291 mV and -284 to -345 mV, respectively. The first notable difference produced by the addition of $H_2S_{(aq)}$ was that EDB was transformed in

Figure 10. Degradation of EDB and partitioning of ^{14}C-products in buffered reducing solutions containing $H_2S_{(aq)}$ as a function of pH and temperature. Each point is the average of duplicate analyses with relative differences less than 3%.

10a. Solutions buffered at pH 5 with an $H_2S_{(aq)}$ concentration of ca. 500μM.



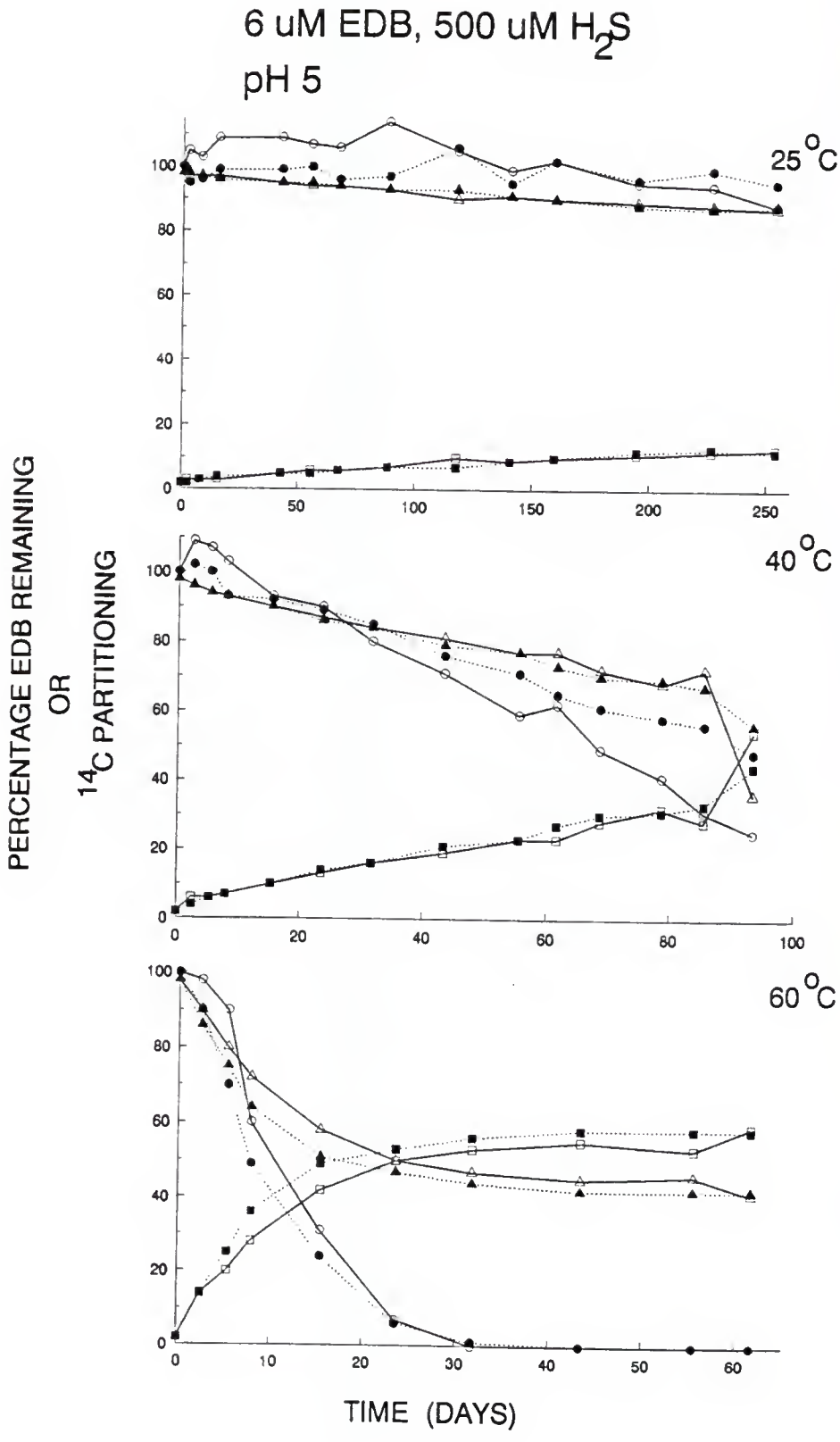
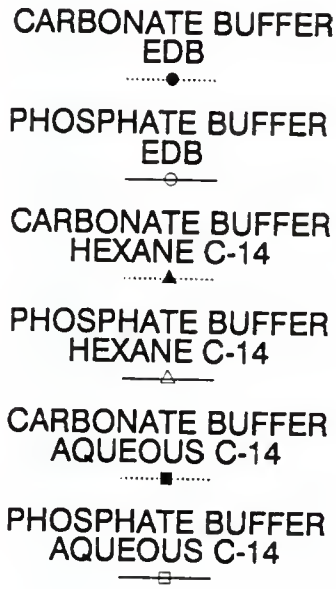

6 uM EDB, 500 uM H_2S
pH 5
25 °C
40 °C
60 °C
PERCENTAGE EDB REMAINING
OR
^{14}C PARTITIONING
TIME (DAYS)

Figure 10 continued.

10b. Solutions buffered at pH 7 with an $H_2S_{(aq)}$ concentration of ca. 500μM.



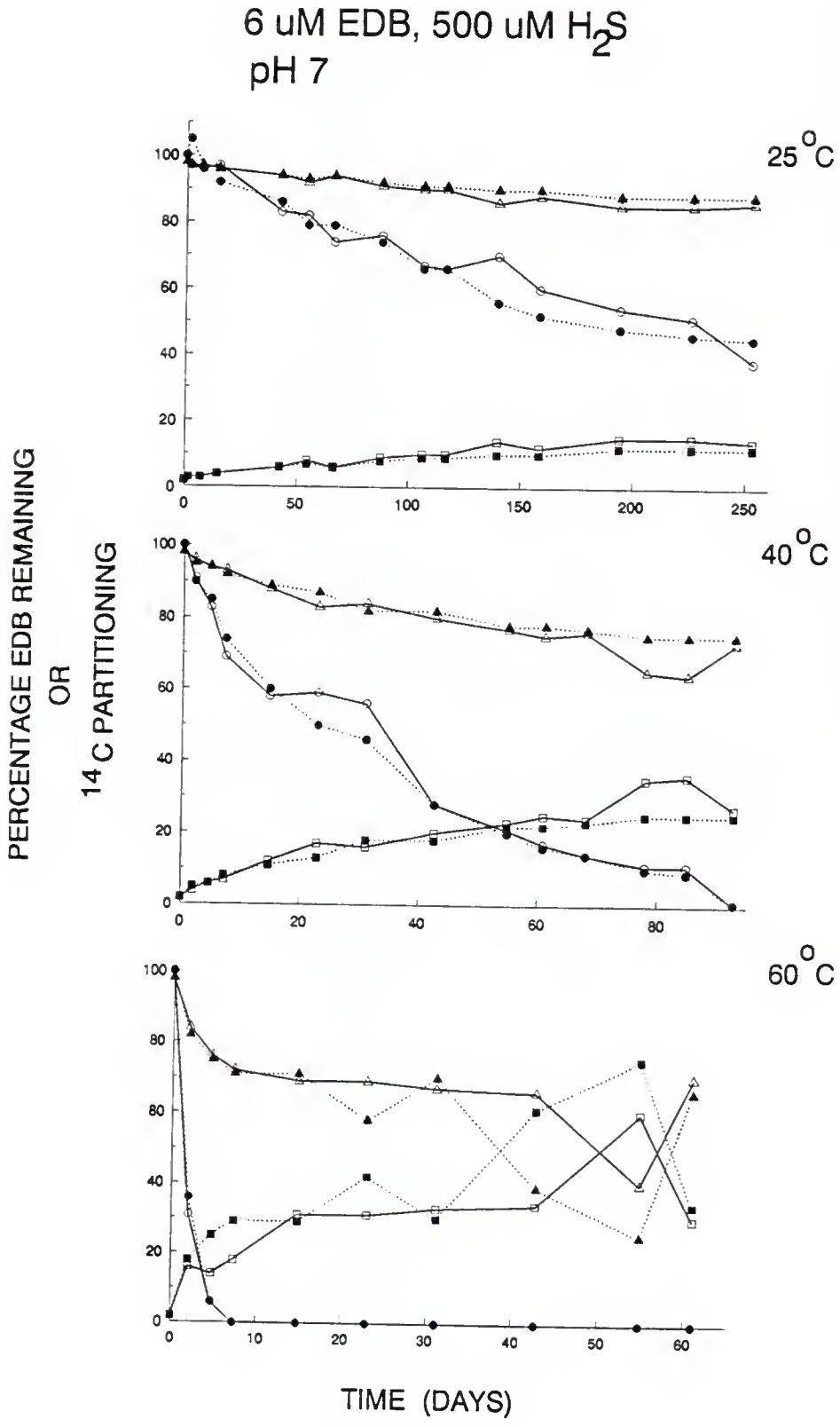
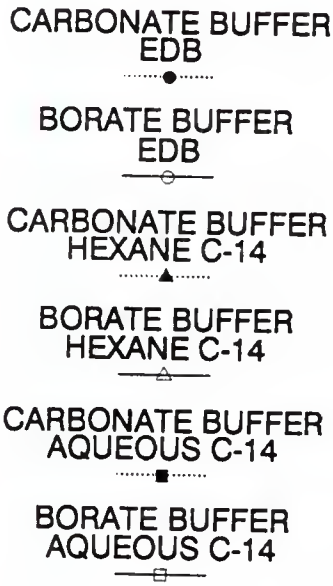

6 uM EDB, 500 uM H_2S
pH 7
25 °C
40 °C
60 °C
PERCENTAGE EDB REMAINING
OR
^{14}C PARTITIONING
TIME (DAYS)

Figure 10 continued.

10c. Solutions buffered at pH 9 with an $H_2S_{(aq)}$ concentration of ca. 500μM.



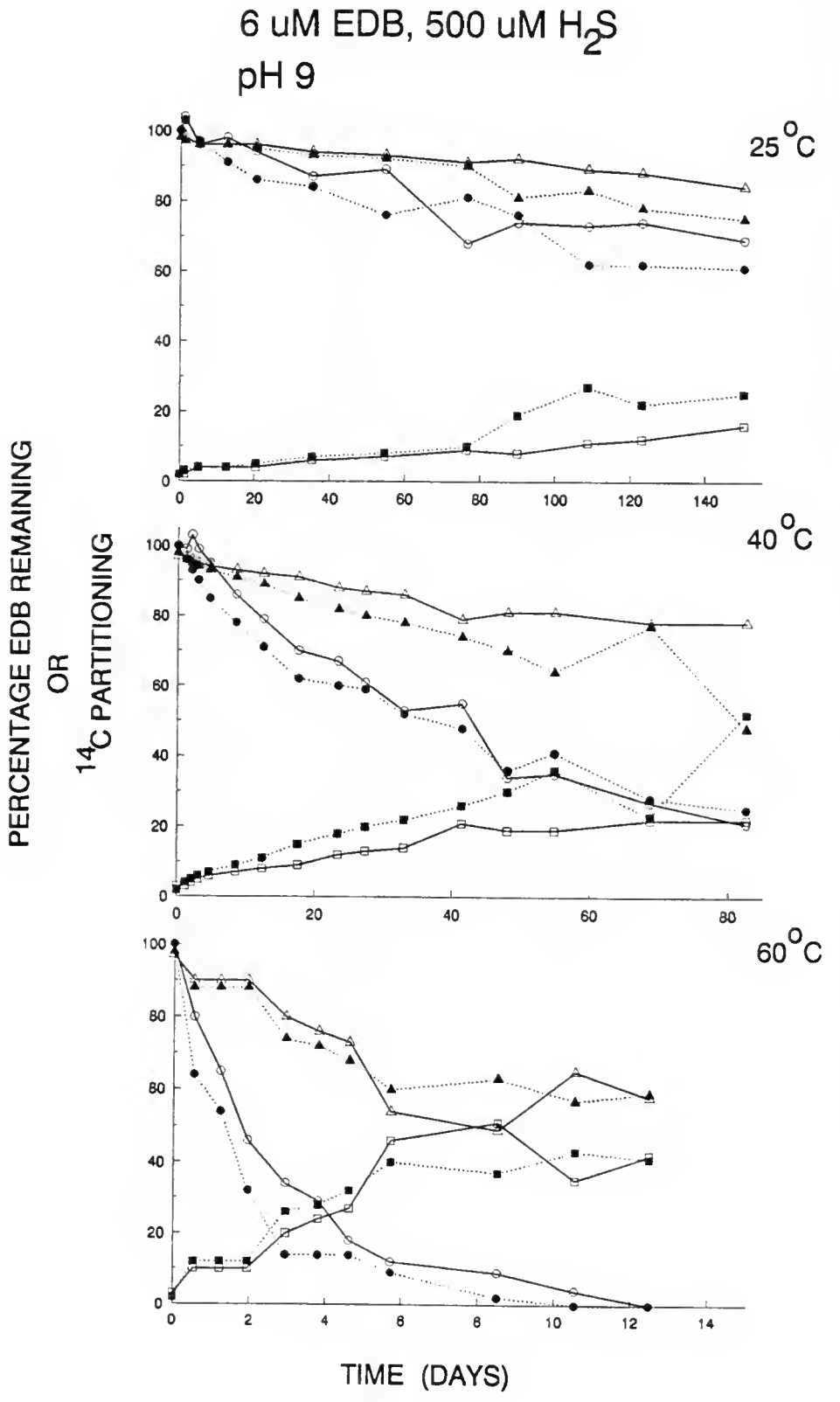
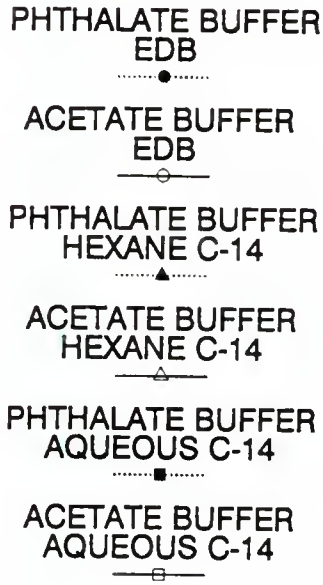

6 uM EDB, 500 uM H_2S
pH 9
25 °C
40 °C
60 °C
PERCENTAGE EDB REMAINING
OR
^{14}C PARTITIONING
TIME (DAYS)

Figure 10 continued.

10d. Solutions buffered at pH 5 with an $H_2S_{(aq)}$ concentration of ca. 1000μM.



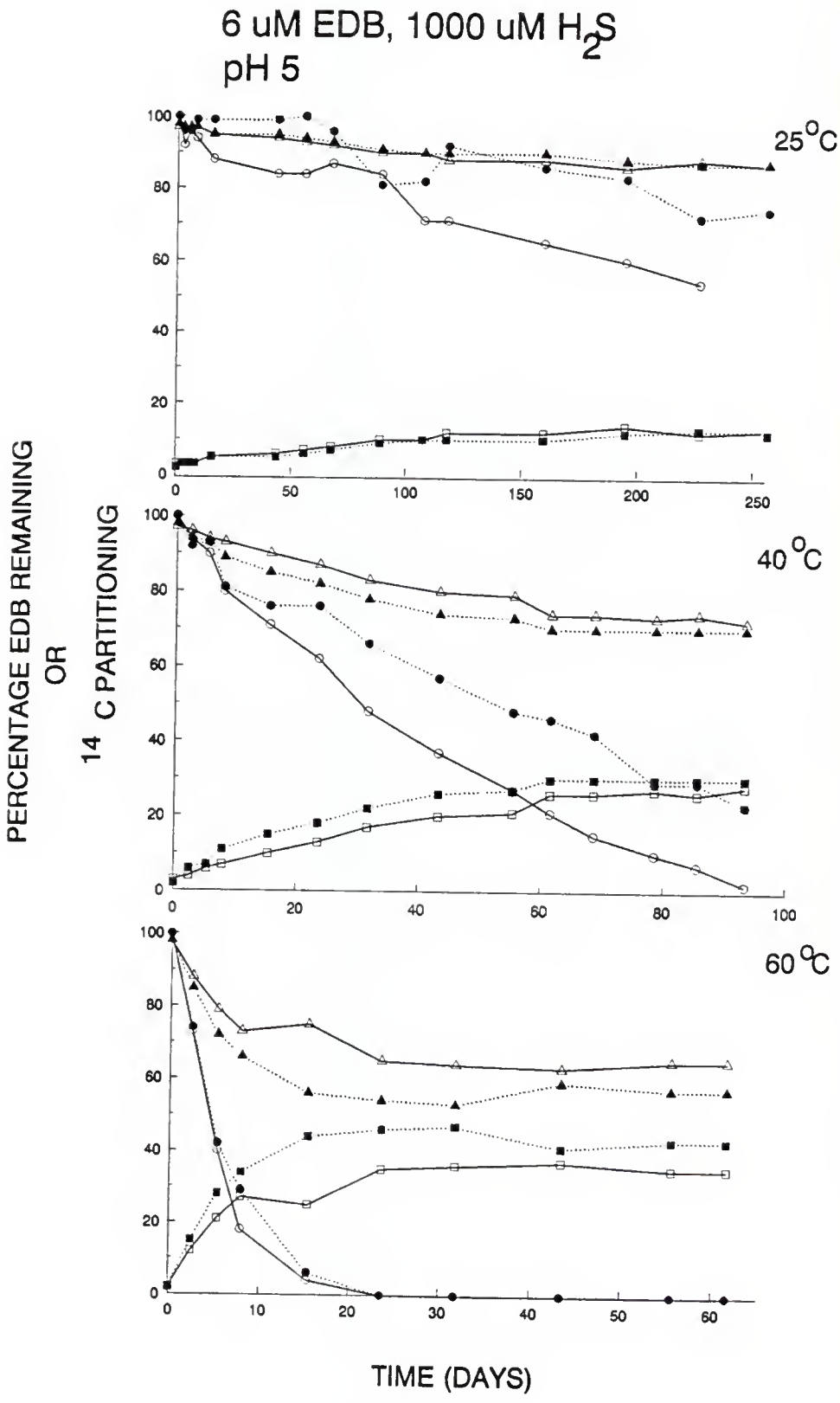
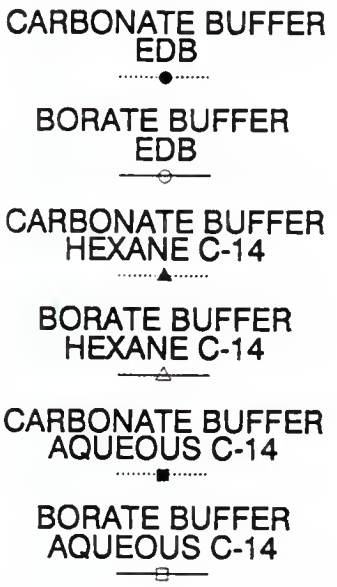

6 uM EDB, 1000 uM H_2S
pH 5
25°C
40°C
60°C
PERCENTAGE EDB REMAINING
OR
^{14}C PARTITIONING
TIME (DAYS)

Figure 10 continued.

10f. Solutions buffered at pH 9 with an $H_2S_{(aq)}$ concentration of ca. 1000μM.



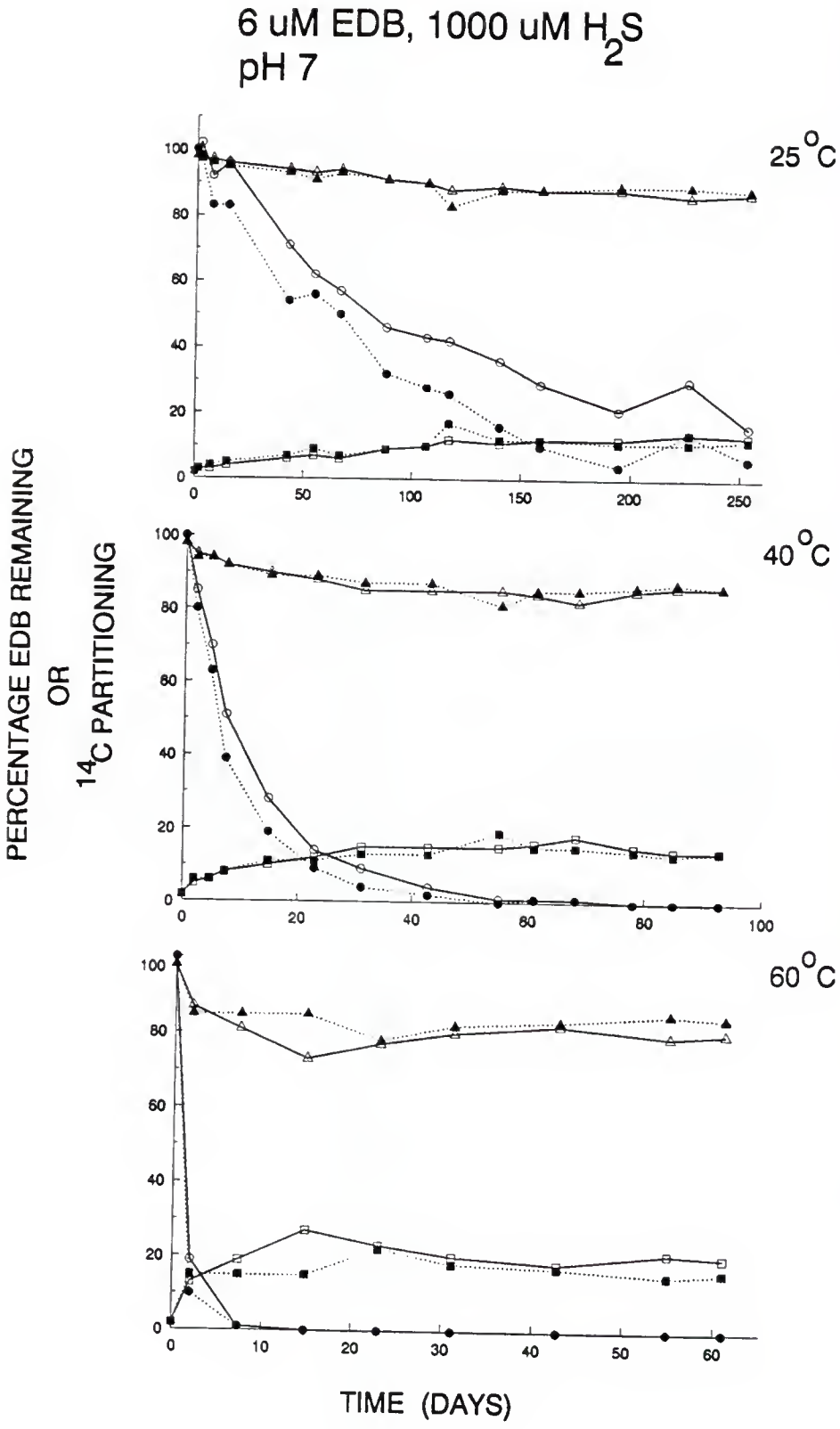
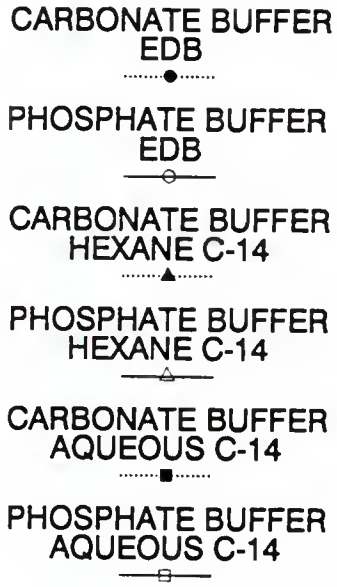
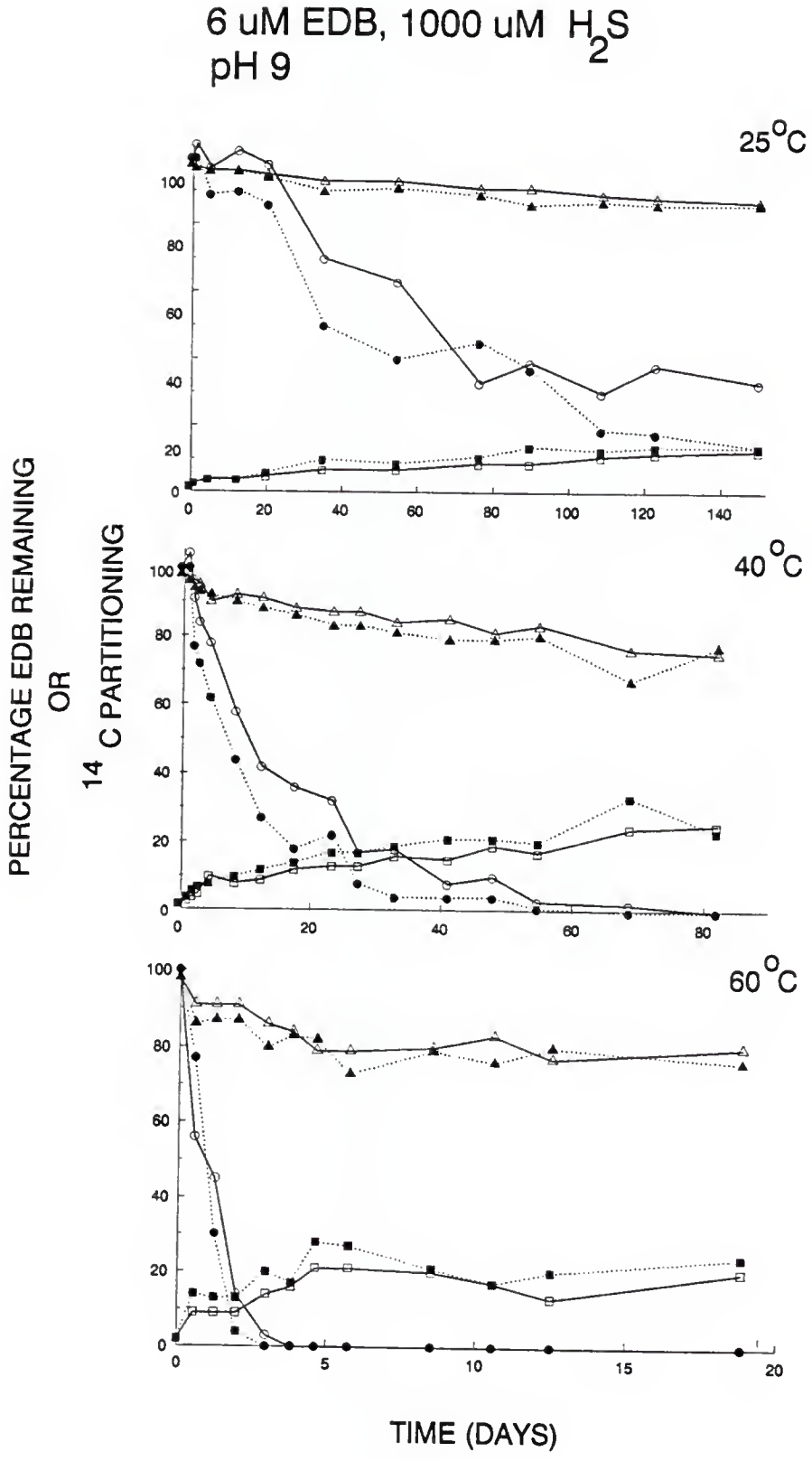

6 uM EDB, 1000 uM H_2S
pH 7
25 °C
40 °C
60 °C
PERCENTAGE EDB REMAINING
OR
^{14}C PARTITIONING
TIME (DAYS)

Figure 10 continued.

10e. Solutions buffered at pH 7 with an $H_2S_{(aq)}$ concentration of ca. 1000μM.




6 uM EDB, 1000 uM H_2S
pH 9
25°C
40°C
60°C
PERCENTAGE EDB REMAINING
OR
^{14}C PARTITIONING
TIME (DAYS)

incubations at a temperature as low as 25°C. Furthermore, degradation rates of EDB were greater at pH 7 and 9 compared to pH 5 at all temperatures. The time at which the initial 6 μM EDB was no longer detected was much shorter in the presence of $H_2S_{(aq)}$, ranging between 32 days (at pH 5, 60°C,and 500 μM $H_2S_{(aq)}$) and 4 days (at pH 9, 60°C, and 1000 μM $H_2S_{(aq)}$).

Secondly, the partitioning of ^{14}C-products in the presence of $H_2S_{(aq)}$ was not into the aqueous phase, as in previous experiments. The recovery of the ^{14}C-activity (initially ^{14}C-EDB tracer) in the individual ampules analyzed during the incubation period was between 95 and 105%. The ^{14}C-activity found in the hexane and aqueous phases after extraction was also between 95 and 105% of the amount of tracer in the original solution.

Apparent first-order disappearance rate constants were derived from plots of the ln percent EDB remaining vs. time for each of the pH- and redox-buffered deionized water control solutions and solutions containing $H_2S_{(aq)}$ (Tables 8, 9, 10). The initial concentrations of EDB (ca. 6 μM) and $H_2S_{(aq)}$ (500 or 1000 μM) were determined immediately after filling and sealing the incubation ampules. In general, greater than half the initial concentration of $H_2S_{(aq)}$ remained by the midpoint of incubations. By the end of the incubations amounts of $H_2S_{(aq)}$ remaining ranged from 65% of the initial concentration to none detectable.

Table 8. Apparent first-order rate constants for the degradation of EDB in solutions at 25, 40 and 60°C and with pH 5 to 9.

Temp (°C)	Buffer[a]/ data pts.	pH[b]	Initial [EDB], μM	Initial [H_2S], μM	10^3 k_{obs}[c] d^{-1}		C.D.[d] (r^2)
25	PHT/6	5.01	5.61	0	-0.33	± 0.30	0.2232
	ACE/6	5.04	8.22	0	-0.20	± 0.26	0.1258
	PHOS/6	6.84	6.35	0	-0.57	± 0.33	0.4329
	CARB/6	7.13	5.62	0	-0.57	± 0.33	0.4356
	BOR/6	9.10	5.47	0	-0.57	± 0.57	0.4959
	CARB/6	8.89	5.78	0	-0.41	± 0.18	0.5742
40	PHT/15	5.01	5.61	0	5.14	± 0.33	0.9490
	ACE/15	5.04	8.22	0	2.82	± 0.26	0.8977
	PHOS/15	6.84	6.35	0	5.40	± 0.26	0.9703
	CARB/15	7.13	5.62	0	3.81	± 0.25	0.9449
	BOR/13	9.10	5.47	0	5.60	± 0.46	0.9320
	CARB/15	8.89	5.78	0	5.53	± 0.26	0.9728
60	PHT/13	5.01	5.61	0	65.02	± 3.78	0.9641
	ACE/13	5.04	8.22	0	62.67	± 2.51	0.9827
	PHOS/13	6.84	6.35	0	61.62	± 3.40	0.9676
	CARB/13	7.13	5.62	0	66.53	± 5.22	0.9366
	BOR/7	9.10	5.47	0	80.45	± 6.02	0.9728
	CARB/13	8.89	5.78	0	68.45	± 2.62	0.9709

[a] Buffers: 0.010 M; PHT= phthalate, ACE= acetate, PHOS= phosphate, CARB= carbonate, BOR= borate; μ= 0.015 M, adjusted with KCl; 0.001 M Ti (III) citrate redox buffer.

[b] pH deviated < 1.0 unit during incubation; change with temperature < 2% (Covington et al, 1985).

[c] Calculated as pseudo-first order reaction with EDB, [H_2O] >> [EDB]. Uncertainties represent the standard error in the slope of the linear least-squares fit plots of ln [EDB] remaining vs. time.

[d] Coefficient of determination; where r is the correlation coefficent in linear least-square regression analysis.

Table 9. Apparent first-order rate constants for the degradation of EDB in solutions containing initial concentrations of ca. 500 μM $H_2S_{(aq)}$ at 25, 40 and 60°C and with pH 5 to 9.

Temp (°C)	Buffer[a]/ data pts.	pH[b]	Initial [EDB], μM	Initial $[H_2S]_T$, μM	10^3 k_{obs}[c] d^{-1}		C.D.[d] (r^2)
25	PHT/14	4.58	5.99	384.2	0.04	± 0.12	0.0119
	ACE/15	5.00	5.45	400.2	0.06	± 0.33	0.2076
	PHOS/15	6.58	5.90	494.6	3.15	± 0.20	0.9517
	CARB13	6.90	5.37	561.2	4.01	± 0.16	0.9837
	BOR/12	9.10	5.96	599.1	2.76	± 0.37	0.8476
	CARB/12	8.95	5.99	637.0	3.04	± 0.41	0.8431
40	PHT/14	4.58	5.99	384.2	7.48	± 0.27	0.9847
	ACE/14	5.00	5.45	400.2	14.02	± 0.10	0.9368
	PHOS/14	6.58	5.90	494.6	32.81	± 3.54	0.8776
	CARB/13	6.90	5.37	561.2	28.20	± 0.60	0.9951
	BOR/16	9.10	5.96	599.1	19.42	± 0.72	0.9810
	CARB/16	8.95	5.99	637.0	16.71	± 0.74	0.9729
60	PHT/6	4.58	5.99	384.2	117.55	± 8.17	0.9811
	ACE/6	5.00	5.45	400.2	182.21	± 15.20	0.9728
	PHOS/3	6.58	5.90	494.6	591.26	± 1.30	1.0000
	CARB/3	6.90	5.37	561.2	606.39	± 45.75	0.9943
	BOR/10	9.10	5.96	599.1	300.43	± 16.28	0.9886
	CARB/10	8.95	5.99	637.0	471.79	± 39.32	0.9770

[a] Buffers: 0.010 M; PHT= phthalate, ACE= acetate, PHOS= phosphate, CARB= carbonate, BOR= borate; μ= 0.015 M, adjusted with KCl; 0.001 M Ti (III) citrate redox buffer.

[b] pH deviated < 1.0 unit during incubation; change with temperature < 2% (Covington et al., 1985).

[c] Calculated as pseudo-first order reaction with EDB, $[H_2S_{(aq)}]$ >> [EDB]. Uncertainties represent the standard error in the slope of the linear least-squares fit plots of ln [EDB] remaining vs. time.

[d] Coefficient of determination; where r is the correlation coefficent in least square linear regression analysis.

Table 10. Apparent first-order rate constants for the degradation of EDB in solutions containing initial concentrations of ca. 1000 μM $H_2S(aq)$ at 25, 40 and 60°C and with pH 5 to 9.

Temp (°C)	Buffer[a]/ data pts.	pH[b]	Initial [EDB], μM	Initial $[H_2S]_T$, μM	10^3 k_{obs}[c] d^{-1}		C.D.[d] (r^2)
25	PHT/15	5.00	5.99	706.1	1.18	± 0.28	0.5806
	ACE/14	5.72	5.85	748.2	2.53	± 0.38	0.7877
	PHOS/15	6.95	5.52	929.3	6.83	± 0.44	0.9488
	CARB/15	7.73	5.34	1193.9	11.54	± 1.07	0.8993
	BOR/12	8.98	5.74	1309.3	9.22	± 1.33	0.8269
	CARB/12	9.05	6.03	1189.7	13.35	± 1.03	0.9439
40	PHT/14	5.00	5.99	706.1	14.35	± 0.68	0.9735
	ACE/13	5.72	5.85	748.2	28.72	± 1.20	0.9811
	PHOS/11	6.95	5.52	929.3	71.22	± 3.84	0.9745
	CARB/11	7.73	5.34	1193.9	78.30	± 9.05	0.8926
	BOR/16	8.98	5.74	1309.3	63.05	± 3.13	0.9666
	CARB/14	9.05	6.03	1189.7	78.10	± 4.41	0.9631
60	PHT/6	5.00	5.99	706.1	357.52	± 65.20	0.8827
	ACE/5	5.72	5.85	748.2	214.19	± 11.43	0.9915
	PHOS/3	6.95	5.52	929.3	988.17	± 67.48	0.9954
	CARB/3	7.73	5.34	1193.9	1074.16	± 43.33	0.9984
	BOR/7	8.98	5.74	1309.3	1746.87	± 185.92	0.9464
	CARB/4	9.05	6.03	1189.7	1624.96	± 343.06	0.9182

[a] Buffers: 0.010 M; PHT= phthalate, ACE= acetate, PHOS= phosphate, CARB= carbonate, BOR= borate; μ= 0.015 M, adjusted with KCl; 0.001 M Ti (III) citrate redox buffer.

[b] pH deviated < 1.0 unit during incubation; change with temperature < 2% (Covington et al., 1985).

[c] Calculated as pseudo-first order reaction with EDB, $[H_2S_{(aq)}]$ >> [EDB]. Uncertainties represent the standard error in the slope of the linear least-squares fit plots of ln [EDB] remaining vs. time.

[d] Coefficient of determination; where r is the correlation coefficent in least square linear regression analysis

Buffered solutions incubated at 25°C (Table 8) had poor coefficients of determination for the least-squares regression line and slopes not significantly different then zero, i.e., a large standard error of the slope compared tothe slope. At 40 and 60°C, coefficients of determination (r^2) ranged from 0.90 to 0.98 and the experimental uncertainty, represented by the standard error of the slope, was no greater than 10 percent in these plots. The linearity of these plots indicate first-order degradation or pseudo-first order hydrolysis kinetics with EDB. In agreement with the experiment carried out in buffered groundwater and deionized water, the degradation rate of EDB in these reducing solution controls (without $H_2S_{(aq)}$) was independent of pH, within experimental error.

In the solutions incubated with $H_2S(aq)$, the EDB degradation first-order plots were linear (i.e., coefficients of determination (r^2) between 0.88 and 1.00), at all three incubation temperatures and all three pH values with one exception. The coefficients of determination ranged from 0.1 to 0.8 for solutions buffered at pH 5 incubated at 25°C, the rate of EDB degradation was indistinguishable from experimental error, i.e., EDB degradation did not occur under these conditions through the duration of the experiment. All other first-order plots had standard errors of the slopes less than 10 percent. The observed EDB degradation rate constants in these solutions with $H_2S_{(aq)}$, ranged from approximately 1.5 to more than 20

times the greater than rate constant for the corresponding pH and temperature control incubation. The effect of the initial concentration of $H_2S_{(aq)}$ on the EDB degradation observed rate constants, k_{obs}, as a function of pH at 40°C is shown in Figure 11. The dashed boxes in the figure correspond to the common initial $H_2S_{(aq)}$ concentrations. Relative to their respective pH values, in control reducing solutions containing concentrations of ca. 500 or 1000 μM $H_2S_{(aq)}$, the EDB degradation rate constants were approximately an order-of-magnitude greater.

The speciation of the total $H_2S_{(aq)}$, $[H_2S]_T$, as $H_2S_{(aq)}$ or HS^- is a function of both pH and temperature. Temperature has a marked effect on the first dissociation constant of $H_2S_{(aq)}$, k_{a1}, as described by Barbero et al. (1982). By measuring apparent molar heat capacities and volumes for NaHS and $H_2S_{(aq)}$ the following expression was derived:

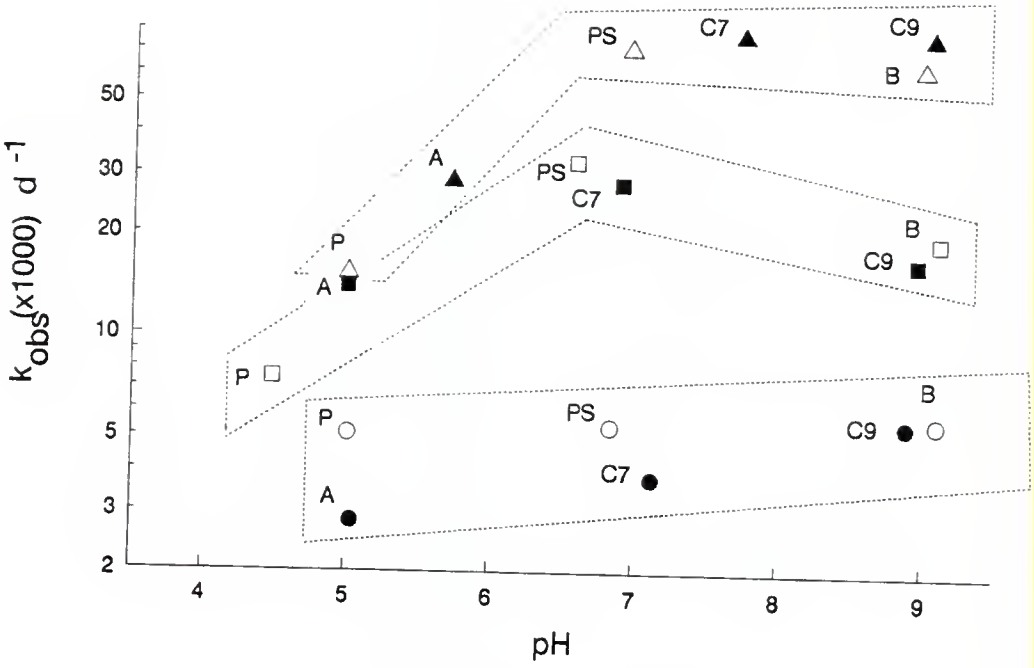
$$\log K_{a1}(T) = 19.840 + 930.8/T - 2.800 \ln T,$$

where $K_{a1}(T)$ is the first-acidic dissociation constant at temperature, T (°K). The resulting temperature-corrected k_{a1} values were derived and the sulfide speciation was determined using the Henderson-Hasselbach equation as follows:

$$pH = pk_{a1} + \log [HS-]/[H_2S_{(aq)}]$$

The calculated values of pk_{a1} for all the incubations are shown in Tables 11 and 12. The $pK_{a\ of}$ second-acidic dissociation constant of $H_2S_{(aq)}$ is 12.90 at 25°C (Dean, 1985). Between pH 5 and 9, the concentration of S^{-2} is very

Figure 11. First-order EDB degradation rate constants as a function of pH, buffer and $H_2S_{(aq)}$ concentration at 40°C.



REDUCING SOLUTION
○ ●

500 uM ca. H2S
□ ■

1000 uM ca. H2S
△ ▲

P = PHTHALATE
A = ACETATE
PS = PHOSPHATE
C7 = CARBONATE
B = BORATE
C9 = CARBONATE

small; at pH 9.0 and 25°C, the concentration of S^{-2} in a solution of 1000 μM $H_2S_{(aq)}$ would be 0.13 μM. Furthermore, the lower concentration of this species present in solution at pH 7 (0.0013 μM) is not reflected in changes in the observed EDB degradation rates. Therefore, it is probably not an important reactant.

The second-order rate constants, k_{H2S} and k_{HS-}, for EDB degradation were then derived (Tables 11 and 12). They were calculated as follows:

$$k_{H2S} = k_{obs}/[H_2S]_T \quad \text{and} \quad k_{HS-} = k_{obs}/[HS-],$$

where $[H_2S]_T$ is the total initial molar concentration of sulfide as $H_2S_{(aq)}$, and [HS-] is the initial molar concentration of sulfide present as HS-.

The values of k_{H2S} are consistent with the corresponding observed first-order rate constants, k_{obs} in Tables 9 and 10. The rate of EDB degradation increases both with temperature and $H_2S_{(aq)}$ concentration. Buffering the solutions at pH 5 resulted in values of k_{H2S} at least one-half less than the values in solutions buffered at pH 7 or 9. On the other hand, the values of k_{HS-} are not consistent with the corresponding first-order rate constants, k_{obs}. The k_{H2S} values from corresponding pH buffers and temperature incubations obtained for the initial concentrations of 500 and 1000 μM $H_2S_{(aq)}$ are in good agreement, within experimental error, exception for solutions buffered at pH 9 and incubated at 60°C. In the latter solutions, k_{H2S} values at

Table 11. Second-order rate constants for degradation of EDB, calculated for initial concentrations of ca. 500 μM $H_2S_{(aq)}$.

Temp (°C)	Buffer[a]	pH[b]	pK_{a1}[c]	Initial $[H_2S]_T$, μM	Initial $[HS^-]$, μM	k_{H2S},[d] $M^{-1}\ d^{-1}$	k_{HS^-},[e] $M^{-1}\ d^{-1}$
25	PHT	4.58	7.01	384.2	1.4	0.12	32.14
	ACE	5.00		400.2	4.0	0.15	15.50
	PHOS	6.58		494.6	241.6	6.38	13.06
	CARB	6.90		561.2	248.6	7.15	16.15
	BOR	9.10		599.1	594.4	4.60	4.60
	CARB	8.95		637.0	629.9	4.77	4.82
40	PHT	4.58	6.73	384.2	2.7	21.48	2770.75
	ACE	5.00		400.2	7.4	35.02	1894.05
	PHOS	6.58		494.6	206.4	66.34	158.97
	CARB	6.90		561.2	336.4	50.26	83.84
	BOR	9.10		599.1	596.6	27.41	32.55
	CARB	8.95		637.0	633.2	26.24	26.40
60	PHT	4.58	6.37	384.2	6.1	304.53	19269.84
	ACE	5.00		400.2	16.3	455.30	11178.59
	PHOS	6.58		494.6	305.7	1195.43	1934.12
	CARB	6.90		561.2	432.7	1080.53	1401.41
	BOR	9.10		599.1	598.0	501.15	502.39
	CARB	8.95		637.0	635.3	740.64	742.63

[a] Buffers: 0.010 M; PHT= phthalate, ACE= acetate, PHOS= phosphate, CARB= carbonate, BOR= borate; μ= 0.015 M, adjusted with KCl; 0.001 M Ti (III) citrate redox buffer.

[b] pH deviated < 1.0 unit during incubation; change in pH with temperature < 2% (Covington et al., 1985).

[c] Temperature dependence of the first ionization constant of $H_2S_{(aq)}$, pK_{a1}, is described by Barbero et al. (1982). Speciation was calculated by the Henderson-Hasselbalch equation, $pH = pk_{a1} + \log [HS^-]/[H_2S_{(aq)}]$.

[d] Second-order rate constant, $k_{obs}/[H_2S_{(aq)}]_T$; $[H_2S_{(aq)}]$ > [EDB].

[e] Second-order rate constant, $k_{obs}/[HS^-]$; $[HS^-]$ > [EDB].

Table 12. Second-order rate constants for degradation of EDB, calculated for initial concentrations of ca. 1000 μM $H_2S_{(aq)}$.

Temp (°C)	Buffer[a]	pH[b]	pK_{a1}[c]	Initial $[H_2S]_T$, μM	Initial $[HS^-]$, μM	k_{H2S},[d] $M^{-1}\,d^{-1}$	k_{HS^-},[e] $M^{-1}\,d^{-1}$
25	PHT	5.00	7.01	706.1	7.0	1.67	261.43
	ACE	5.72		748.2	37.3	3.38	67.77
	PHOS	6.95		929.3	459.3	7.35	14.87
	CARB	7.73		1193.9	1006.5	9.67	11.47
	BOR	8.98		1309.3	1295.8	7.04	7.12
	CARB	9.05		1189.7	1179.2	11.22	11.32
40	PHT	5.00	6.73	706.1	13.1	29.33	1095.57
	ACE	5.72		748.2	67.3	38.38	426.70
	PHOS	6.95		929.3	582.4	76.64	122.29
	CARB	7.73		1193.9	1086.0	65.60	72.10
	BOR	8.89		1309.3	1302.0	48.15	48.42
	CARB	9.05		1189.7	1184.1	65.65	65.96
60	PHT	5.00	6.37	706.1	28.7	506.33	12436.02
	ACE	5.72		748.2	136.1	286.27	1573.78
	PHOS	6.95		929.3	734.7	1063.35	1344.99
	CARB	7.73		1193.9	1143.7	899.71	939.20
	BOR	8.98		1309.3	1306.0	1334.20	1337.57
	CARB	9.05		1109.3	1187.1	1464.87	1368.85

[a] Buffers: 0.010 M; PHT= phthalate, ACE= acetate, PHOS= phosphate, CARB= carbonate, BOR= borate; μ= 0.015 M, adjusted with KCl; 0.001 M Ti (III) citrate redox buffer.

[b] pH deviated < 1.0 unit during incubation; change in pH with temperature < 2% (Covington et al., 1985).

[c] Temperature dependence of the first ionization constant of $H_2S_{(aq)}$, pK_{a1}, is described by Barbero et al. (1982). Speciation was calculated by the Henderson-Hasselbalch equation, $pH = pk_{a1} + \log [HS^-]/[H_2S_{(aq)}]$.

[d] Second-order rate constant, $k_{obs}/[H_2S_{(aq)}]_T$; $[H_2S_{(aq)}]$ > [EDB].

[e] Second-order rate constant, $k_{obs}/[HS^-]$; $[HS^-]$ > [EDB].

1000 μM $H_2S_{(aq)}$ exceed values with 500 μM $H_2S_{(aq)}$ by a factor of two or three. In addition, at all temperatures and all $H_2S_{(aq)}$ concentrations, the k_{H2S} values at pH 7 were greater than those at pH 9. These kinetic observations suggest that the reactions involved in the degradation of EDB in presence of $H_2S_{(aq)}$ do not simply involve the attack of HS^- on the EDB molecule.

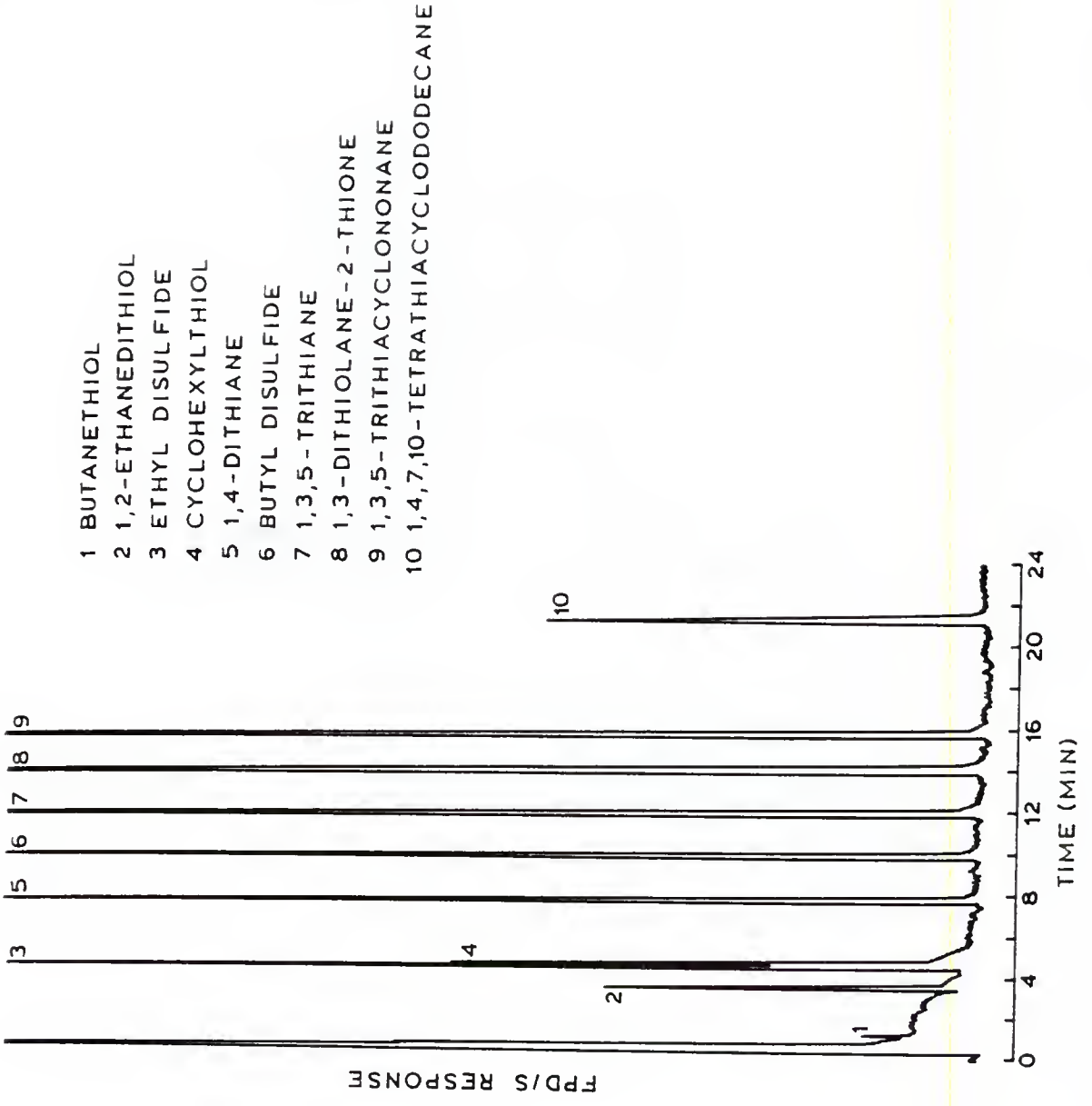
Product Identification

In an effort to identify the majority of the products resulting from the transformation of EDB in solutions with $H_2S_{(aq)}$, initial concentrations of 60 μM EDB and 1000 or 2000 μM $H_2S_{(aq)}$ in buffered solutions were incubated and examined as described before in the kinetic experiments.

The number and size of peaks in GC/EC chromatograms of hexane extracts increased during the progression of the kinetic experiments. Experiments employing greater initial reactant concentrations for the purpose of identifying transformation products, however, were more informative. Use of GC/FPD-S provided information for selectively identifying sulfur-containing products, while GC/MS gave valuable compound structural information.

To aid in identifying the sulfur-containing transformation products of EDB, conditions were established for separation and detection of ten standard sulfur-containing compounds described earlier (Table 4). In Figure 12, the resulting chromatogram of these analytical standards is shown. Linear ranges of detection of these compounds

Figure 12. Gas chromatogram showing separation and FPD-S detection of 10 commercially available sulfur-containing standards used in identifying sulfur-containing products from the reaction of EDB and $H_2S_{(aq)}$.


1 BUTANETHIOL
2 1,2-ETHANEDITHIOL
3 ETHYL DISULFIDE
4 CYCLOHEXYLTHIOL
5 1,4-DITHIANE
6 BUTYL DISULFIDE
7 1,3,5-TRITHIANE
8 1,3-DITHIOLANE-2-THIONE
9 1,3,5-TRITHIACYCLONONANE
10 1,4,7,10-TETRATHIACYCLODODECANE
FPD/S RESPONSE
TIME (MIN)
0
4
8
12
16
20
24

established under these conditions were from 0.10 nmol to 5.00 nmol (equivalent to 8 to 1100 ng) for each compound injected.

The sulfur-containing compound, 1,2,5,6-tetrathiacyclooctane, not commercially available, was synthesized following the procedure of Goodrow et al. (1982). After recrystallization from hexane, most of the product melted between 90-95°C. Some non-melting solid remained in the melt up to 300°C. Goodrow et al. (1982) reported similar observations. With the GC/FPD-S and GC/MS techniques used in this study, the purity of product after the hexane recrystallization was adequate for product identification. Single peaks were present in the GC/FPD-S chromatograms of both the starting material, 1,2-ethanedithiol, and the synthesis product (Figure 13). A single product peak was also obtained in the GC/MS analysis and provided evidence supporting the fact that the synthesis was successful. This will be discussed later.

Mass spectra, in both electron impact (EI) and chemical ionization (CI) modes helped in identifying the EDB transformation products formed in the solutions with H_2S(aq). The mass spectra of the sulfur-containing standards introduced earlier (Table 4 and Figure 12) are presented in Table 13. In the EI spectrum of each compound, the molecular ion (ionized, intact ion M^+) was present; it was the most intense peak (i.e., the base peak, assigned

Figure 13. Gas chromatogram, using FPD-S detection, of solvent blank (recrystallization solvent, 1,2-ethanedithiol (the precuror for the organic synthesis), 1,2,5,6-terathiacyclooctane, the product.

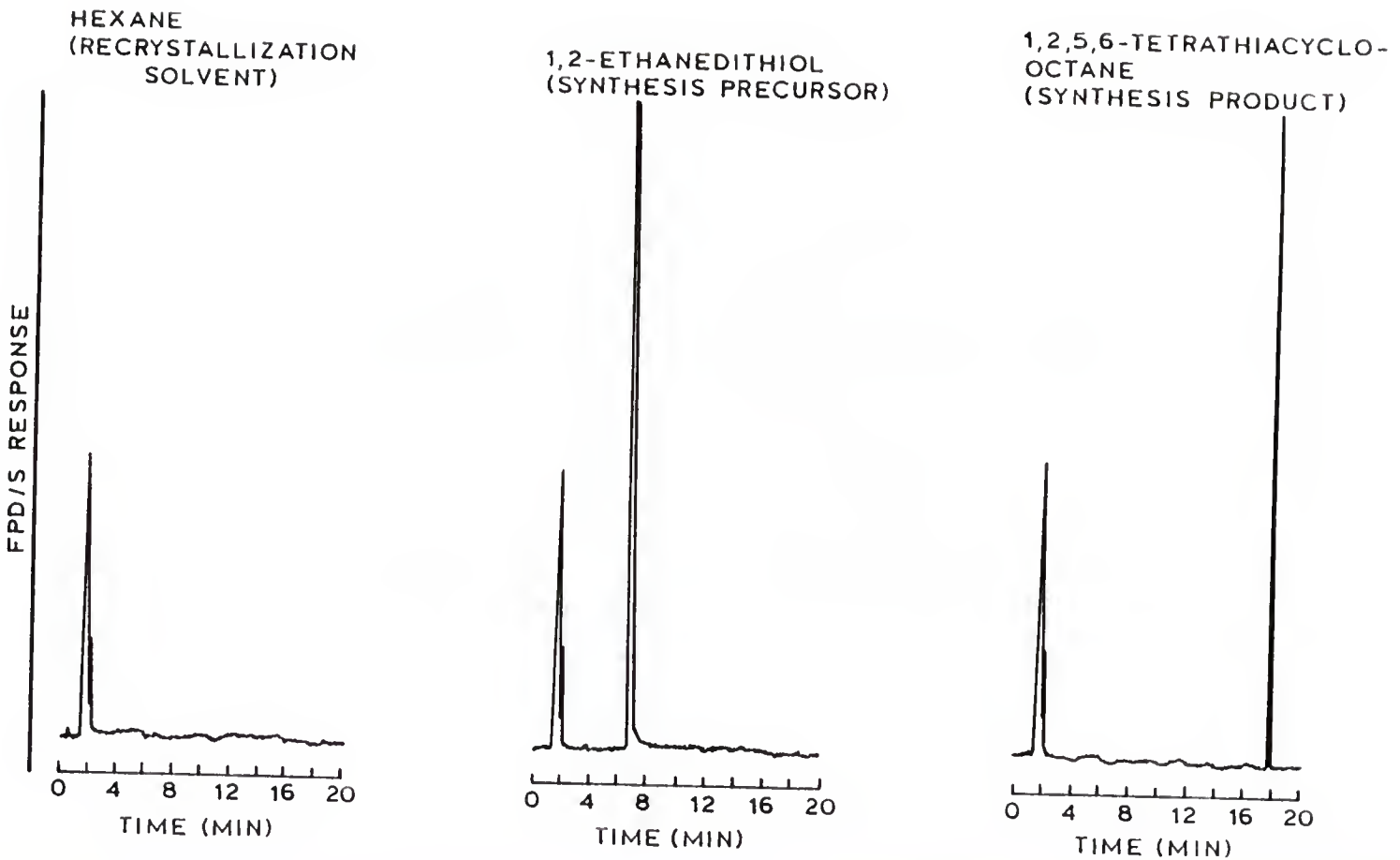

HEXANE
(RECRYSTALLIZATION
SOLVENT)
1,2-ETHANEDITHIOL
(SYNTHESIS PRECURSOR)
1,2,5,6-TETRATHIACYCLO-
OCTANE
(SYNTHESIS PRODUCT)
FPD/S RESPONSE
0 4 8 12 16 20
TIME (MIN)
0 4 8 12 16 20
TIME (MIN)
0 4 8 12 16 20
TIME (MIN)

Table 13. Mass spectrometry of sulfur-containing standard compounds.

Compound	Chemical Formula/ Formula Weight	Fragment Ions, m/z (Relative Intensity)[a]		S Isotope Peak Ratio[d]; M+2		Adduct Ions, CI
		Electron Impact Mode[b]	Chemical Ionization Mode[c]	Observed EI, CI	Calculated (# of S Atoms)	
1-Butanethiol	$C_4H_{10}S$ 90.16	90 (65), 71 (18), 61 (20), 56 (100), 47 (45), 44 (21), 39 (44), 36 (11)	N.A.	N.D.	4.4 (1)	N.D.
1,2-Ethanedithiol	$C_2H_6S_2$ 94.20	96 (6), 94 (75), 73 (18), 61 (36), 60 (57), 59 (28), 58 (24), 57 (25), 47 (100), 45 (40)	N.A.	8.1	8.8 (2)	N.D.
Cyclohexylthiol	$C_6H_{12}S$ 116.23	116 (54), 83 (54), 82 (56), 81 (7), 73 (8), 67 (69), 60 (10), 59 (6), 58 (8), 57 (18), 56 (15), 55 (100), 54 (28), 53 (11)	173 (3), 119 (5), 118 (8), 117 (100), 116 (15)	3.7, 5.0	4.4 (1)	M + 57
Ethyl disulfide	$C_4H_{10}S_2$ 122.25	124 (8), 123 (6), 122 (100), 94 (59), 68 (7), 66 (89), 64 (10), 61 (8), 60 (11), 59 (14), 58 (10), 57 (17), 56 (11)	N.A.	8.0	8.8 (2)	N.D.
1,4-Dithiane	$C_4H_{10}S_2$ 120.24	122 (7), 121 (7), 120 (100), 92 (15), 73 (7), 64 (11), 61 (50), 60 (23), 59 (12), 58 (12), 55 (6)	176 (1), 163 (4), 161 (2), 123 (11), 122 (8), 121 (100), 120 (8), 117 (2)	7.3, 8.4	8.8 (2)	M + 43 M + 57
1,3-Dithiolane-2-thione	$C_3H_4S_3$ 136.26	138 (11), 137 (5), 136 (100), 108 (7), 76 (28), 64 (16), 60 (41), 59 (7), 44 (35)	N.A.	10.8	13.2 (3)	N.D.
1,3,5-Trithiane	$C_3H_6S_3$ 138.27	140 (13), 138 (100), 92 (28), 91 (9), 86 (13), 73 (9), 64 (16), 61 (6), 60 (12), 59 (10), 58 (6), 57 (70), 56 (45), 55 (7)	N.A.	11.5	13.2 (3)	N.D.
Butyldisulfide	$C_8H_{18}S_2$ 178.36	178 (39), 122 (33), 87 (6), 86 (6), 58 (9), 57 (100), 56 (19), 55 (9)	267 (2), 235 (2), 221 (3), 181 (9), 180 (12), 179 (100), 178 (16)	N.D., 8.5	8.8 (2)	M + 43 M + 57

(Continued)

(Continued) Table 13.

Compound	Chemical Formula/ Formula Weight	Fragment Ions, m/z (Relative Intensity)[1]		S Isotope Peak Ratio[4]; M+2		Adduct Ions, CI
		Electron Impact Mode[2]	Chemical Ionization Mode[3]	Observed EI, CI	Calculated (# of S Atoms)	
1,4,7-Trithiacyclo-nonane	$C_6H_{12}S_3$ 180.36	182 (9), 181 (11), 180 (100), 121 (27), 120 (22), 119 (27), 106 (42), 105 (16), 93 (11), 92 (13), 87 (16), 85 (9), 78 (15), 64 (28), 61 (95), 60 (46), 59 (42), 58 (26), 57 (11), 47 (16)	237 (1), 183 (13), 182 (10), 181 (100), 179 (12), 153 (11), 135 (6), 121 (12), 106 (11)	9.1, 12.5	13.2 (3)	M + 57
1,2,5,6-Tetrathiacyclo-octane	$C_4H_8S_4$ 184.28	186 (18), 184 (100), 156 (7), 130 (9), 128 (51), 126 (9), 124 (73), 96 (8), 93 (8), 92 (58), 91 (7), 66 (5), 64 (54), 61 (11), 60 (24), 59 (36), 58 (13), 57 (7)	N.A.	18.0	17.6 (4)	---
1,4,7,10-Tetrathiacyclo-dodecane	$C_8H_{16}S_4$ 240.47	240 (47), 121 (41), 120 (35), 105 (35), 87 (41), 86 (35), 64 (29), 61 (59), 60 (71), 59 (35), 46 (53), 45 (59), 44 (59), 43 (29), 40 (100)	297 (2), 243 (17), 242 (13), 241 (100), 240 (15), 213 (17), 195 (7), 153 (9), 135 (5), 121 (8), 106 (12)	N.D., 17.4	17.6 (4)	M + 57

[a] Percent of the base ion peak; ions with greater than 5% relative intensity shown for electron impact, greater than 1% shown for chemical ionization.

[b] Source temperature, ca. 250 °C; 0.2 Torr; electron energy, 70 eV

[c] Positive ion mode; isobutane ionizing reagent gas; source temperature, ca. 150 °C; electron energy, 70 eV

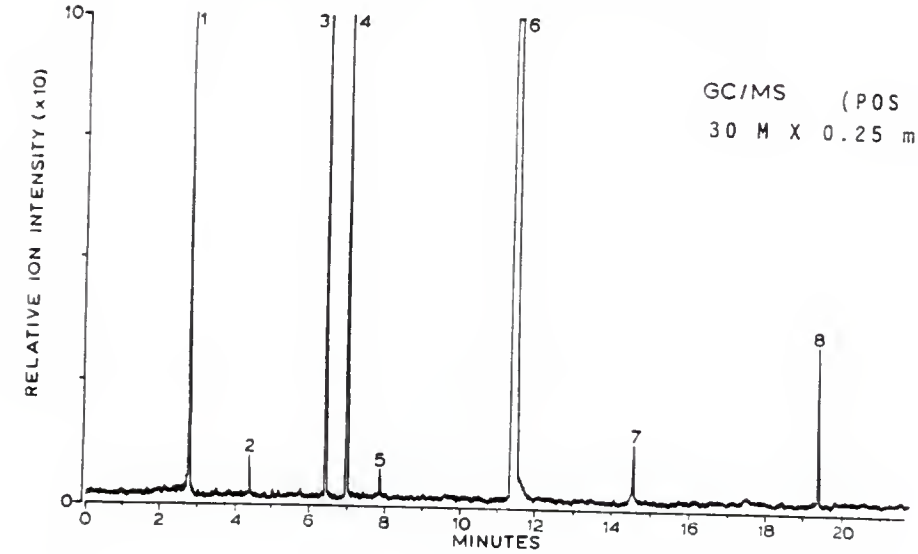
[d] S-isotope ratio of molecular ion (M) or pseudo-molecular ion (M+1) peaks shown in EI and CI spectra, respectively; natural abundance ^{34}S = 4.4%

N.A. Not analyzed in this mode

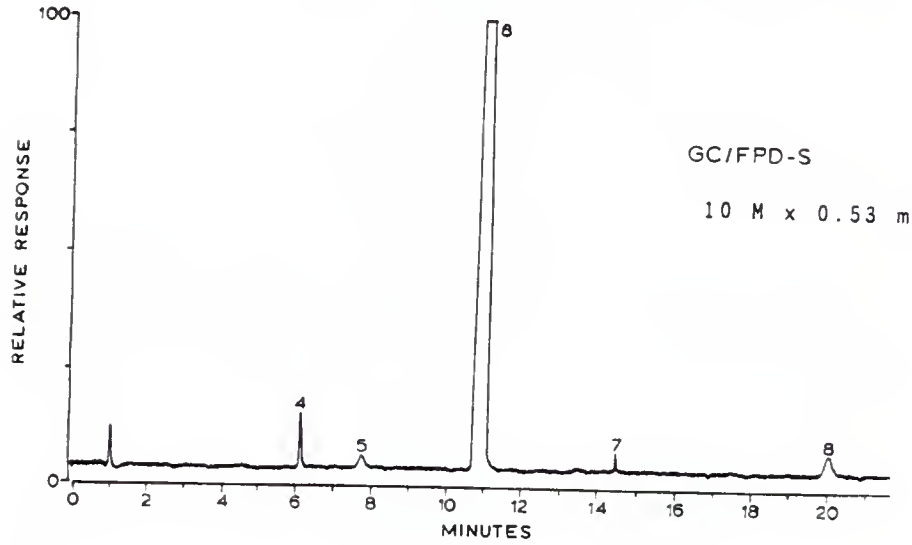
N.D. Not detected

a value of 100%) in 5 of the 11 standards. In the CI spectra, pseudo-molecular ions (M+1) were always present as the base peak. The sulfur isotope ratios observed for the molecular ions, i.e., M+2/M for the natural occurrence of $^{34}S:^{32}S$, 4.4:100, were generally very close to the calculated values (Table 13). CI spectra containing M+43 and M+57 were observed. These are the adduct ions produced by addition of the isobutane ionization reagent gas. The adduct ions are useful for confirming molecular weights of the standards and unknowns. The reconstructed GC/MS chromatogram and the GC/FPD-S chromatogram of a hexane extract of the reaction solution (Figure 14), gave peaks with similar retention times. One (1,4-dithane) of the five sulfur-containing standard compounds, chromatographed under the same GC/FPD-S conditions, matched the retention time of a peak in the extract chromatogram. Mass spectra of well resolved peaks detected in the extracts of several EDB and $H_2S_{(aq)}$ incubations are compiled in Table 14. In all EI mass spectra, the molecular ion peak (M^+, ionized intact compound) was the most intense ion fragment, i.e., the base peak assigned a value of 100%, with the exception of the 1,4,7,10-tetrathiacyclododecane (Table 12). In the latter spectra, the molecular ion was somewhat stable, being 39% of the m/e 57 ion base ion peak. All EI spectra were representative of each standard with regard to its true molecular weight. The spectra provided satisfactory

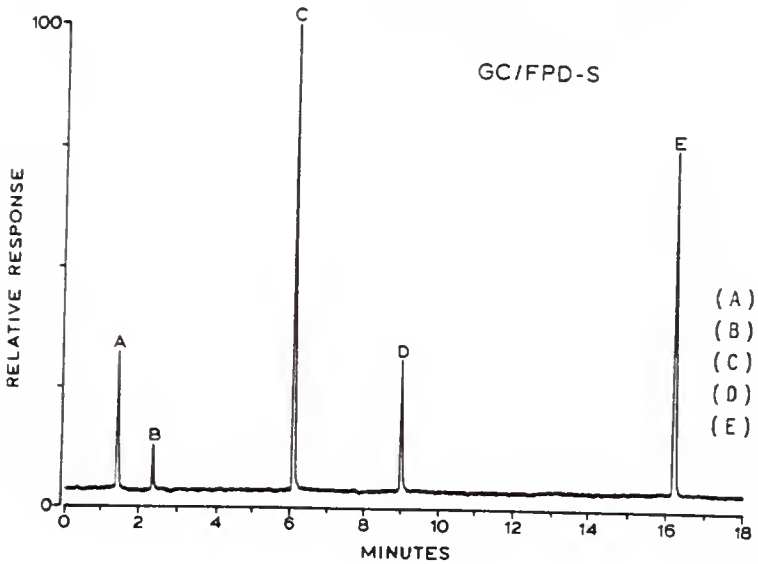
Figure 14. Gas chromatograms of EDB transformation products from solutions containing $H_2S_{(aq)}$ in product studies (top and middle) and sulfur-containing standards (bottom). The legend for numbered peaks in the reconstructed ion chromatogram of positive ion chemical ionization (top) with assigned identities is as follows: 1=EDB, 4=DT, 5=TTCP, 6=TTCH, 7=TTCO, 8=TTCD.



GC/MS (POS CI)
30 M X 0.25 mm (OB-1)



GC/FPD-S
10 M x 0.53 mm (RSL-300)



GC/FPD-S

(A) 1,2-ethanedithiol
(B) cyclohexyl mercaptan
(C) 1,4-dithiane
(D) butyl disulfide
(E) 1,4,7-trithiacyclononane

Table 14. Mass spectrometry structure elucidation of sulfur-containing products in EDB and $H_2S_{(aq)}$ solutions.

Compound	Chemical Formula/ Formula Weight	Fragment Ions, m/z (Relative Intensity)[a]		S Isotope Peak Ratio[d]; M+2		Adduct Ions
		Electron Impact Mode[b]	Chemical Ionization Mode[c]	Observed EI, CI	Calculated (# of S Atoms)	
1,4-Dithiane	$C_4H_{10}S_2$ 120.24	122 (7), 120 (100), 92 (17), 73 (5), 64 (11), 61 (64), 60 (22), 59 (9), 58 (8), 47 (7), 46 (58), 45 (28)	177 (1), 163 (3), 161 (1), 123 (8), 122 (7), 121 (100), 120 (17), 119 (3)	6.6, 6.9	8.8 (2)	M + 43 M + 57
1,2,3-Trithiacyclopentane	$C_2H_4S_3$ 124.20	126 (11), 124 (100), 96 (26), 64 (10), 60 (40), 59 (26), 45 (16)	137 (3), 127 (13), 125 (100), 124 (33), 105 (5)	11.1, 12.6	13.2 (3)	N.D.
1,3-Dithiolane-2-thione	$C_3H_4S_3$ 136.26	138 (14), 136 (100), 108 (14), 107 (7), 81 (7), 76 (21), 59 (21), 45 (21), 40 (14)	N.A.	14.3	13.2 (3)	N.D.
1,3,5-Trithiane	$C_3H_6S_3$ 138.27	140 (13), 138 (100), 92 (28), 91 (9), 86 (13), 73 (9), 64 (16), 61 (6), 60 (12), 59 (10), 58 (6), 57 (70), 56 (45), 55 (7)	195 (2), 149 (2), 140 (5), 139 (100), 138 (23), 135 (5), 107 (12)	12.3, 10.3	13.2 (3)	M + 57
1,2,5-Trithiacycloheptane	$C_4H_8S_3$ 152.22	154 (14), 153 (6), 152(100), 124 (20), 106 (15), 105 (5), 96 (7), 92 (17), 87 (38), 78 (18), 64 (17), 61 (10), 66 (44), 59 (7), 58 (10), 45 (27)	209 (1), 195 (2), 155 (14), 154 (9), 153 (100), 152 (18), 151 (1), 125 (2), 124 (1), 119 (2)	13.5, 13.5	13.2 (3)	M + 43 M + 57
1,2,3,4-Tetrathiacyclo-hexane	$C_3H_4S_4$ 156.26	158 (13), 156 (100), 130 (6), 128 (53), 92(10), 64 (32)	N.A.	13.4	17.6 (4)	N.D.
1,2,5,6-Tetrathiacyclo-octane	$C_4H_8S_4$ 184.28	186 (11), 185 (7), 184 (100), 156 (7), 151 (7), 130 (9), 128 (20), 126 (11), 124 (40), 96 (7), 94 (9), 93 (7), 92 (24), 72 (7), 64 (16), 60 (7), 59 (16), 45 (7)	187 (12), 186 (7), 185 (100), 184 (17), 135 (2), 133 (3), 125 (3), 124 (4), 107 (2)	11.1, 12.3	17.6 (4)	N.D.

(Continued)

(Continued) Table 14.

Compound	Chemical Formula/ Formula Weight	Fragment Ions, m/z (Relative Intensity)		S Isotope Peak Ratio; M+2		Adduct Ions
		Electron Impact Mode	Chemical Ionization Mode	Observed EI, CI	Calculated (# of S Atoms)	
1,4,7,10-Tetrathiacyclo-dodecane	$C_8H_{16}S_4$ 240.47	240 (47), 121 (41), 120 (35), 105 (35), 87 (41), 86 (35), 64 (29), 61 (59), 60 (71), 59 (35), 46 (53), 45 (59), 44 (59), 43 (29), 40 (100)	297 (1), 279 (2), 244 (1), 243 (17), 242 (10), 241 (100), 240 (12), 223 (2), 215 (3), 214 (2), 213 (18), 195 (5), 193 (2), 185 (2), 181 (14), 180 (2), 167 (2), 165 (1), 163 (2), 167 (1), 154 (1), 153 (4), 135 (4), 133 (3), 121 (5), 120 (4), 119 (3), 117 (2), 105 (2), 103 (11), 101 (5)	N.D., 17.0	17.6 (4)	M + 57

[a] Percent of the base ion peak; ions with greater than 5% relative intensity shown for electron impact, greater than 1% shown for chemical ionization.

[b] Source temperature, ca. 250 °C; 0.2 Torr; electron energy, 70 eV

[c] Positive ion mode; isobutane ionizing reagent gas; source temperature, ca. 150 °C; electron energy, 70 eV

[d] S-isotope ratio of molecular ion (M) or pseudo-molecular ion (M+1) peaks shown in EI and CI spectra, respectively; natural abundance ^{34}S = 4.4%

N.A. Not analyzed in this mode

N.D. Not detected

intensity of the M^+ ion and informative fragmentation ions useful for structural confirmation and elucidation of closely related compounds. The pseudo-molecular ions (MH^+ or molecular ion mass + 1, M+1) were present in the positive CI mass spectra of all the standards, and were the base ion peak supporting the molecular weight determined in EI mass spectra. The sulfur-isotope ratio was also used in elucidating structures. When the M^+ ion is strong enough, the number of sulfur atoms present in a compound can be determined by the contribution of the ^{34}S isotope (4.4%). The sulfur-isotope peak ratios (M+2/M+) for the standards are generally in reasonable agreement with the predicted (calculated) values for the number of S atoms in the molecule (Table 13). Lower than expected ratios are probably due insufficient sensitivity to relatively low concentrations of M^+ present while the spectra were obtained. Additional evidence for supporting the molecular weights determinations was provided by reagent gas adduct ions in positive CI mass spectra. The C_4H_9 adduct (M+57) and the C_3H_7 adduct (M+43) were present in the spectra of four standards. The EI mass spectra compiled in Tables 13 and 14 are available in Appendix C.

Assignment of molecular weight and structure to sulfur-containing transformation products in solutions of EDB and $H_2S_{(aq)}$ were made by comparing the mass spectral evidence from several of the product study incubations to the mass

spectral data of the sulfur-containing standards (Table 14). These data provided direct evidence for identification of five of the sulfur-containing compounds (DT, DTT, TT, TTCO, TTCD) present in the extracts of incubations (Table 15). The mass spectra of these products were essentially identical to their corresponding analytical standard. The retention times in GC/MS and GC/FPD-S analyses of these compounds were identical to standards under the same chromatographic conditions. Coinjection of incubation extracts and standards resulted in identical retentions times (Figure 15). Those peaks not matching a standard still had very similar retention times to the standards. The identities of these compounds were based on mass spectral determination of molecular weight. Structural rationale for some of the key fragment ions of TTCP, TTCH and TTCO is presented in Figure 16. Goodrow et al. (1982) note the presence of an intense m/e 124 fragment as confirmation of the identity of TTCO. Since no analytical standards were available for comparison, the identities of TTCP and TTCH are tentative. Compounds with higher molecular weights had longer retention times in the capillary GC analyses. This sequence of elution resembled chromatographic behavior of a homologous series of compounds of similar structure. GC retention indices for closely sulfur-containing compounds have been used for identifying sulfur vesicants and related compounds elsewhere (D'agostino et al. 1988).

Table 15. Chemical and structural formulae of sulfur-containing products from reactions of EDB and $H_2S_{(aq)}$. The identity of these compounds were established by GC/MS and GC retention times of known standards.

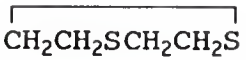
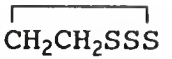
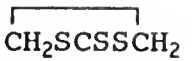
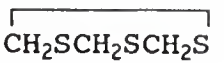
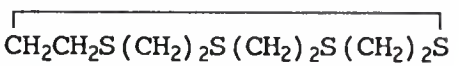
COMPOUND	ABBREVIATION	CHEMICAL FORMULA/ FORMULA WEIGHT	STRUCTURAL FORMULA
1,2-Ethanedithiol	EDT	$C_2H_6S_2$ 94.20	$HSCH_2CH_2SH$
1,4-Dithiane	DT	$C_4H_8S_2$ 120.24	
1,2,3-Trithiacyclopentane	TTCP	$C_2H_4S_3$ 124.20	
1,3-Dithiolane-2-thione	DTT	$C_3H_4S_3$ 136.26	
1,3,5-Trithiane	TT	$C_3H_6S_3$ 138.27	
1,2,5-Trithiacycloheptane	TTCH	$C_4H_8S_3$ 152.22	
1,2,5,6-Tetrathiacyclooctane	TTCO	$C_4H_8S_4$ 184.28	
1,4,7,10-Tetrathiacyclododecane	TTCD	$C_8H_{16}S_4$ 240.47	

Figure 15. Sample GC/FPD-S chromatogram of the sulfur-containing standards (top) and extracted incubation mixture of EDB and $H_2S_{(aq)}$ (middle). Coinjection of standards and incubation extract show superimposing peaks of TT at 12 min and TTCO and 16 min (bottom).

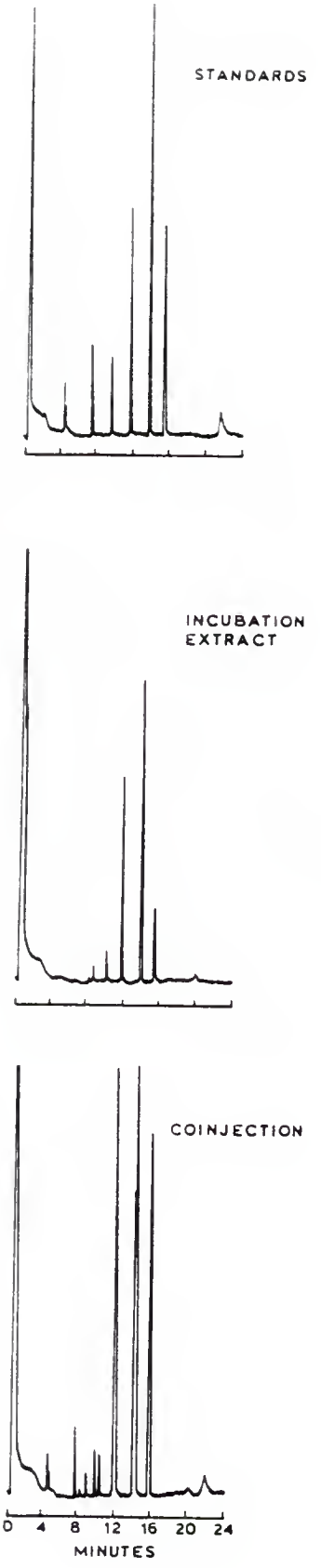
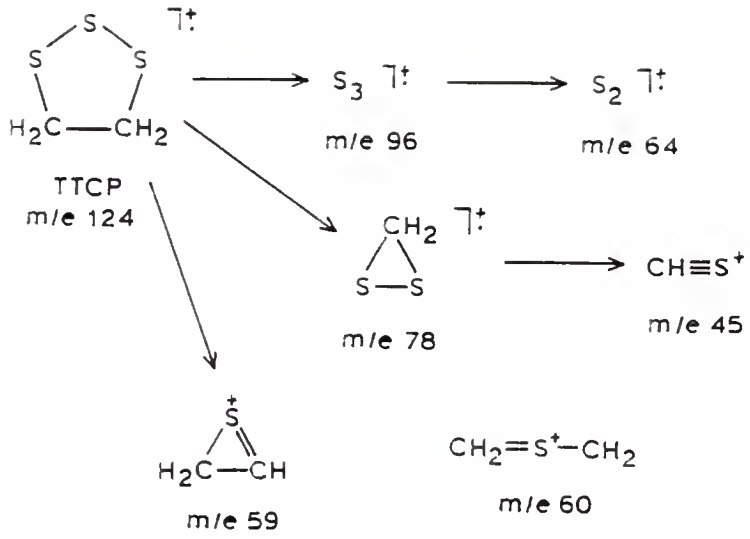
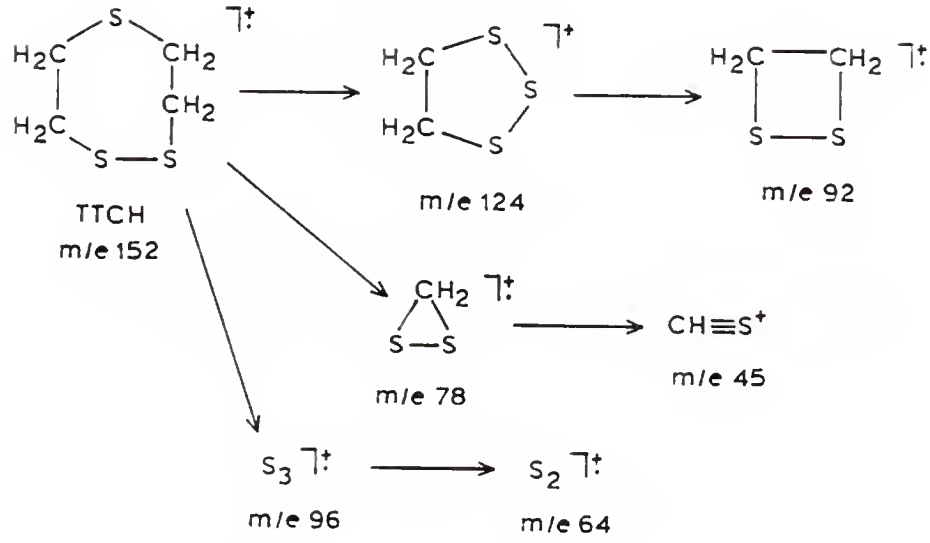
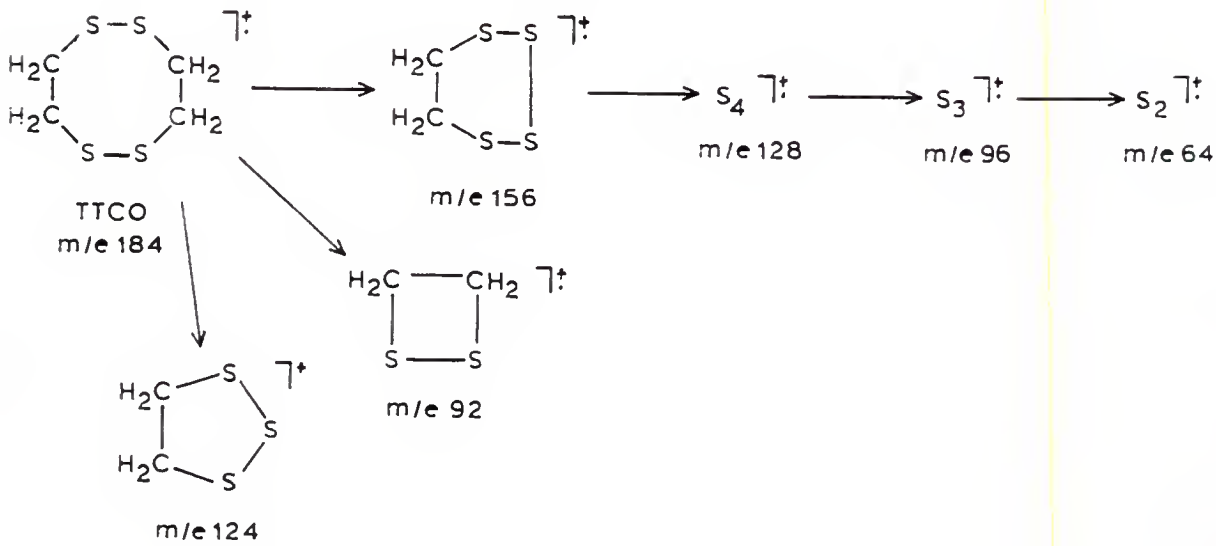

STANDARDS
INCUBATION
EXTRACT
COINJECTION
0 4 8 12 16 20 24
MINUTES

Figure 16. Structural rationale for some key fragment ions in mass spectrometry electron impact fragmentation of sulfur-containing transformation products of EDB.


TTCP
m/e 124
m/e 96
m/e 64
m/e 78
m/e 45
m/e 59
m/e 60


TTCH
m/e 152
m/e 124
m/e 92
m/e 78
m/e 45
m/e 96
m/e 64


TTCO
m/e 184
m/e 156
m/e 128
m/e 96
m/e 64
m/e 92
m/e 124

Ethylene Dibromide in Sulfate Groundwater

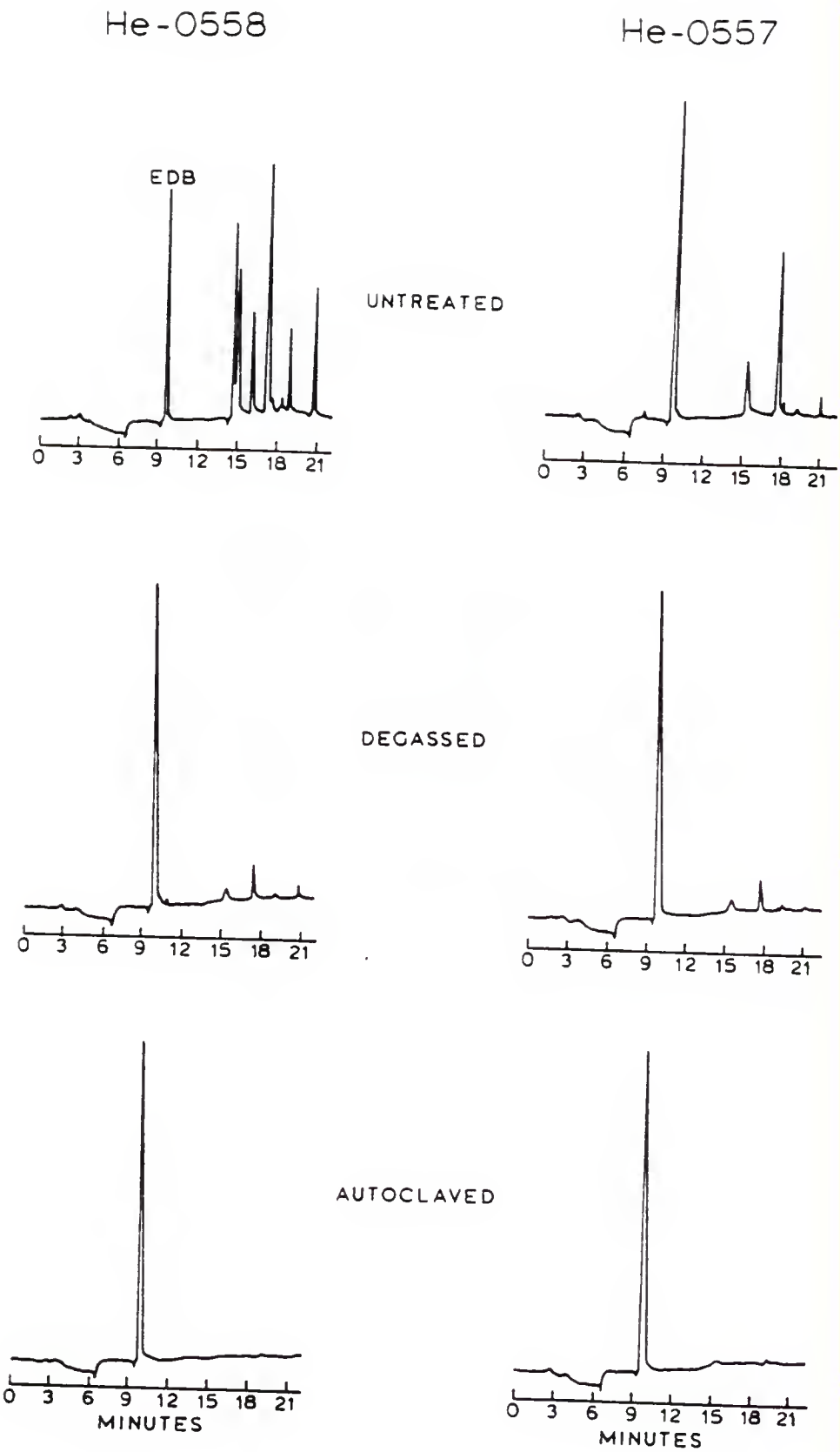
Groundwater with a natural potential for producing $H_2S_{(aq)}$ was chosen in an attempt to observe transformation of EDB by the $H_2S_{(aq)}$ alternative degradation route. Groundwater from four wells in Hendry County and south central Florida were sampled because of historic data of "high sulfate", i.e., concentrations in the range of 200-400 mg/L (Appendix B). The reduction of 200 mg/L of sulfate would produce 71 mg/L (2080 μM) $H_2S_{(aq)}$. The actual groundwater samples, which were fortified with EDB and then studied, came from wells which tapped different depths of the underlying aquifer (Table 16). With regard to reactants with $H_2S_{(aq)}$, one notable difference between the samples was the presence of dissolved iron (Fe) and total Fe. The formation of iron sulfides are common reactions in anaerobic reducing groundwater.

GC/EC chromatograms of the EDB-fortified water samples after storage in the dark at 25°C for 9 months are shown in Figure 17. In the untreated samples, the initial EDB concentration of 1.0 mg/L had decreased in HE-0057 by 40%, HE-0058 by 60%, and by 95% in L-02531. The EDB concentrations in the autoclaved samples had not changed. The sulfur-containing compounds EDT, TT, and TTCO were detected in HE-0558 incubations by GC/FPD-S (Figure 18). This sample was originally fortified, as in the earlier buffered laboratory water product identification study, to

TABLE 16. Florida Ambient Groundwater Monitoring Network sampling results for the water used in experiments in this study.

SITE ID	Sampling DATE	Temp (°C)	pH Units	UMHOS /CM	SO_4 (mg/L)	Fe (mg/L)	Tot Fe (mg/L)	Aquifer	Total Well Depth (Feet)
HE-0557	022387	25.1	7.5	4490	357.7	0.01	0.11	Intermed.	100
HE-0558	022387	24.4	7.1	3780	226.0	5.54	7.35	Surficial	014
L-01977	010587	23.4	7.7	1991	226.5	0.05	0.05	Intermed.	185
L-02531	010587	26.7	7.8	3150	385.9	0.05	0.05	Floridian	605

Figure 17. GC chromatograms of extracts of EDB fortifed groundwater that was incubated for 9 months at 25°C.


He-0558
He-0557
EDB
UNTREATED
DEGASSED
AUTOCLAVED
0 3 6 9 12 15 18 21
MINUTES

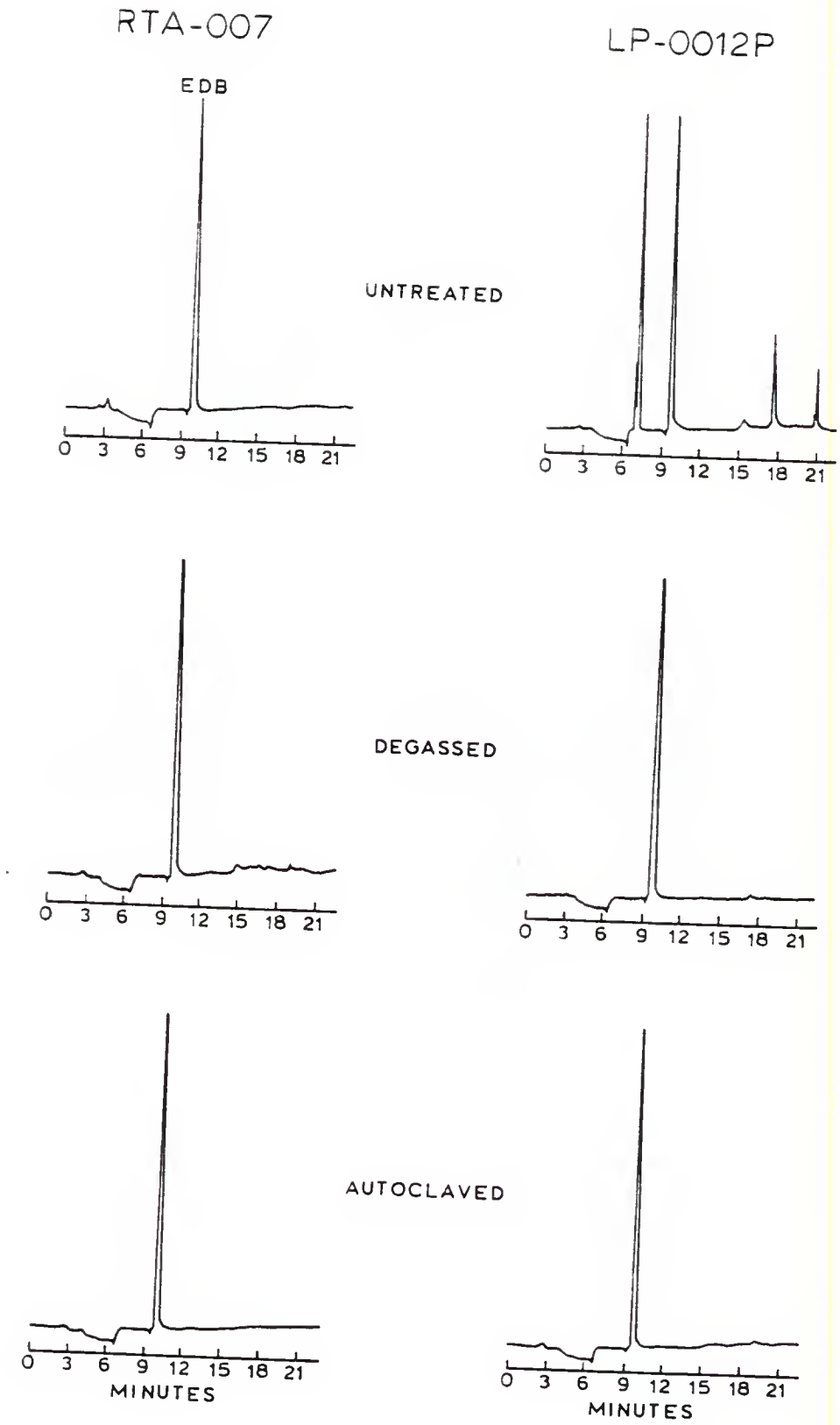
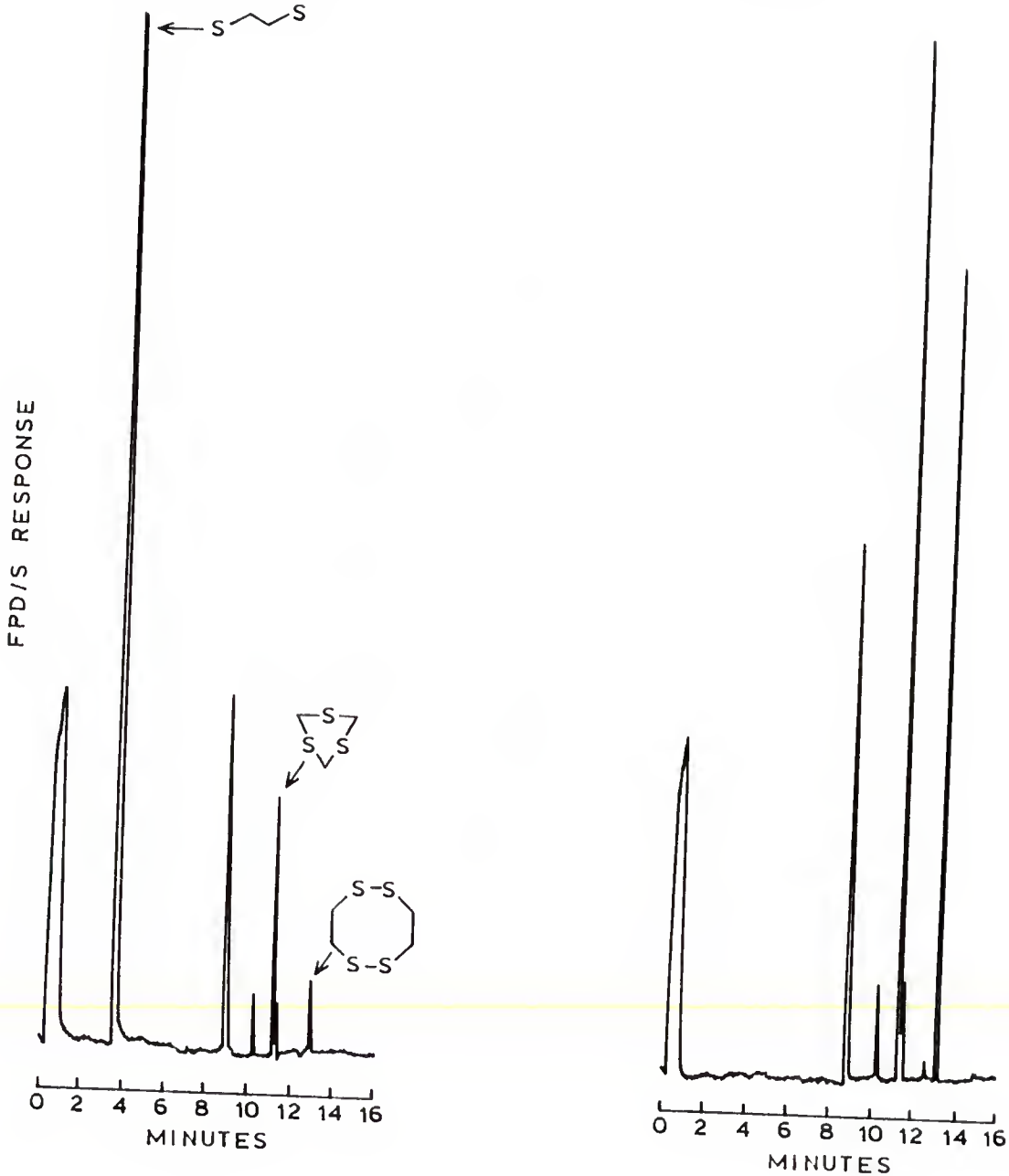

RTA-007
LP-0012P
EDB
UNTREATED
DEGASSED
AUTOCLAVED
0 3 6 9 12 15 18 21
MINUTES

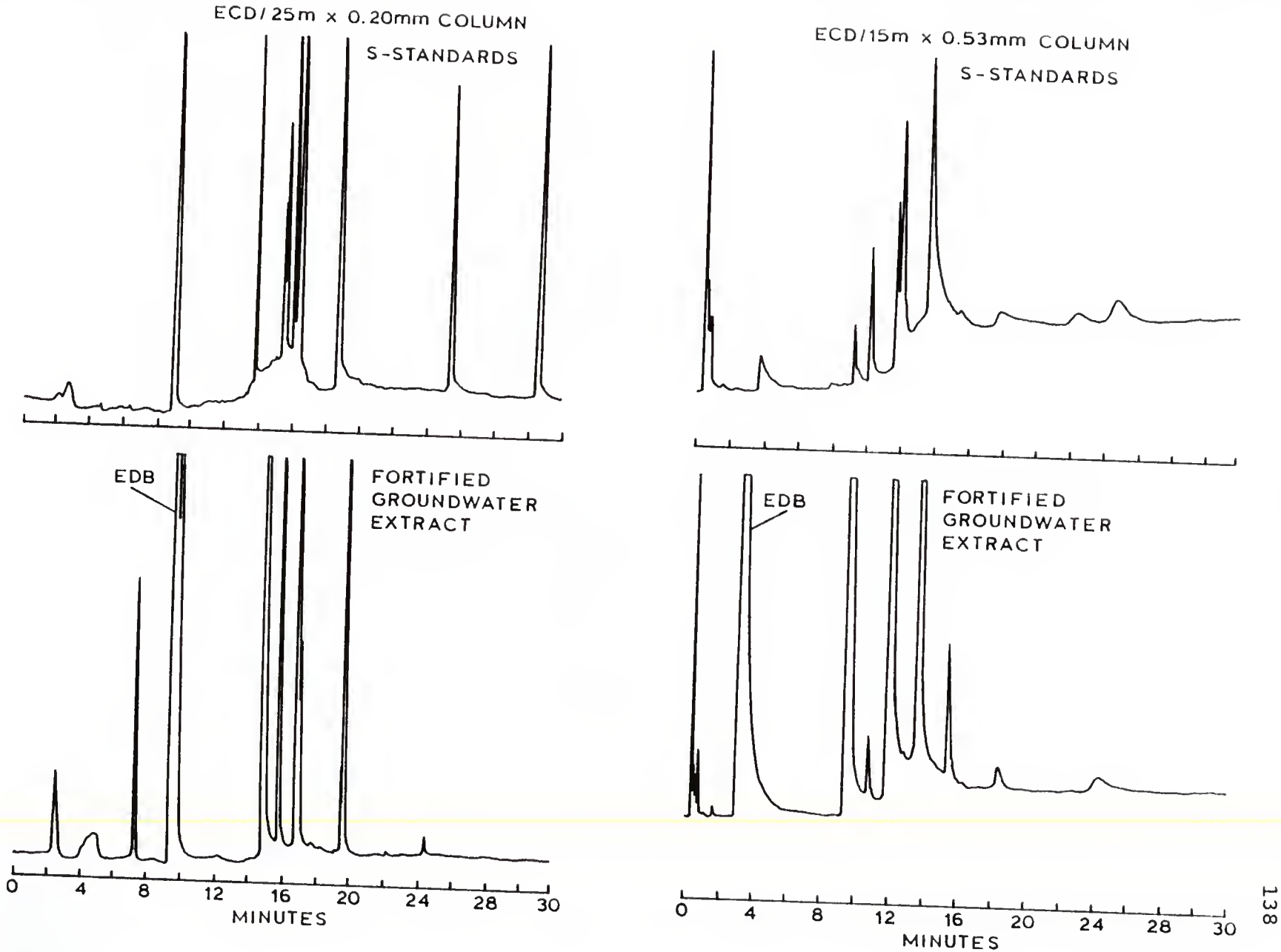
Figure 18. GC chromatograms of extracted groundwater containing naturally occurring $H_2S_{(aq)}$ and incubated anaerobically with EDB at 10 mg/L. Three confirmed sulfur-containing products are identified.


FPD/S RESPONSE
S
S
S
S
S
S-S
S-S
0 2 4 6 8 10 12 14 16
MINUTES
0 2 4 6 8 10 12 14 16
MINUTES

a resulting concentration of 10 mg/L EDB. After storage of the extract in a tightly sealed GC sample vial, reanalysis revealed the EDT peak disappeared and other product peaks increased. Analysis of the sample by GC/EC on two different GC columns shows the difficulty experienced using EC detection for analysis (Figure 19). The chromatograms were not helpful in product identification. Because EC detection is especially sensitive to organohalides, peaks in these chromatograms may be intermediate degradation products containing bromide.

HE-0558 groundwater EDB-fortified samples incubated for more than a year contained $H_2S_{(aq)}$ concentrations of 73 mg/L (2060 μM). In addition, visible quantities of the black precipitate were present, presumably ferrous sulfide (FeS, pyrite).

Figure 19. GC chromatograms comparing two chromatographic columns on a gas chromatograph with an electron capture detector. Upper panels are injections of sulfur-containing standards; bottom panels are injections of an extract of EDB-fortified groundwater incubation (same sample in Figure 18).


ECD/25m x 0.20mm COLUMN
S-STANDARDS
EDB
FORTIFIED
GROUNDWATER
EXTRACT
0
4
8
12
16
20
24
28
30
MINUTES
ECD/15m x 0.53mm COLUMN
S-STANDARDS
EDB
FORTIFIED
GROUNDWATER
EXTRACT
0
4
8
12
16
20
24
28
30
MINUTES

DISCUSSION

Analytical Methods

The sensitive analytical methodologies employed in this study seeked to explore the degradation and transformation of EDB in aqueous systems and groundwater at EDB concentrations which might be found at contaminated sites. The methodology was supported by GC with selective GC detectors, chemical derivatization, mass spectrometry, and the liquid scintillation counting of ^{14}C-labeled analyte. GC/EC analysis of EDB was very sensitive. On the other hand, GC/EC was not successful for the analysis of sulfur-containing compounds, but GC/FPD-S was. The value of monitoring ^{14}C-activity was to (1) detect loss of ^{14}C-analyte throughout incubations and extractions, and (2) to study to partitioning of the transformation products from water into organic extracts. Although not employed in these experiments, thin layer chromatography of ^{14}C-transformation products would be useful to isolate individual compounds to then study their stability and toxicty.

Hydrolysis Reactions

The term "transformation" is commonly used to encompass any changes in the chemical structure of the compound being

studied. A chemical contaminant or pesticide in the environment is transformed by chemical, photochemical and biochemical routes. The persistence of an organic contaminant in the saturated zone, i.e., saturated soil or groundwater, would largely be a function of whether microogranisms can transform the compound. In contrast, transformation by chemical hydrolysis alone is much slower. In my preliminary studies, EDB's disappearence from groundwater at environmental ambient temperature was negligible over several months. Similarly, no significant degradation of EDB was observed in soil at the same time as the water samples which were obtained from the same regions of Florida (Weintraub et al., 1985). It might be expected that the long residence time of EDB in selected Florida soils would allow adequate time for microbes to metabolize EDB; however that did not occur. On the other hand, hydrolytic mechanisms do take place, although at a slower rate than biochemial degradation catalyzed by microbes.

Hydrolytic reactions of EDB were independent of pH between 5 and 9. Therefore, the observed degradation rate constant, k_{obs}, is equivalent to k_n (neutral) in this pH range. In the general acid/base catalysis expression:

$$k_T = k_H[H^+] + k_n + k_{OH}[OH^-]$$

k_H, the rate constant for specific-acid catalysis, and k_{OH}, the rate constant for specific-base catalysis are insignificant. Beyond the environmental pH range, the

degradation rate increases, e.g., at pH 14, a 1.0 mg/L aqueous solution degraded completely in less than 10 days at 23°C. These findings are in agreement with data compiled on the hydrolysis of the general class of compounds of organic halides (Maybey et al., 1978; Harris, 1982). For the purpose of predicting degradation kinetics at environmental pHs, the pseudo-first rate constant for $k_n[H_2O]$, k_h is written for the hydrolysis rate law:

$$-\frac{d[EDB]}{dt} = k_h[EDB]$$

The temperature dependence of any discrete chemical process, i.e., no change in mechanism over the temperature tested, may be expressed by the Arrhenius equation:

$$k = Ae^{-Ea/RT}$$

where A is the entropy term, E_a is the activation energy, R is the gas constant 1.987 cal deg^{-1} mol^{-1}, and T the absolute temperature. The Arrhenius plots (ln k vs 1/T) for EDB degradation were linear, supporting the earlier finding of a single dominant degradation mechanism. A change in the slope reflects a change in the E_a, which is characteristic of a change in the reaction taking place. Extrapolating the experimental rate constants from elevated temperatures in Arrhenius plots to 22°C, the average temperature of Florida groundwater, resulted in rate constants ranging from 6.4 x 10^{-2} to 2.76 x 10^{-3} d^{-1}, corresponding to half-lives of 772 to 259 days. As pointed out by Mabey and Mill (1978), a random

error of 10% in k leads to an E_a accurate only to 100%, or a factor of 2. The uncertainty in E is magnified when experimental rate data are extrapolated over 25°C or longer intervals when making estimates of k under environmental conditions. Due to inherent error in such determinations, it is best to regard rate data obtained in this manner as order-of-magnitude estimates (Harris, 1982).

Reactions with Aqueous Hydrogen Sulfide

The presence of $H_2S_{(aq)}$ in solutions increased the degradation rates of EDB by 3 to 20 fold compared to hydrolysis reactions. The products are distinctly different and much more varied as compared to the hydrolysis reaction. The enhanced reactivity of $H_2S_{(aq)}$ over the oxygen analog, H_2O, stems from aspects of chemistry of the sulfur atom. The electrons of sulfur are more numerous and further removed from the nucleus than those surrounding oxygen; therefore, sulfur's electrons are more polarizable. When sulfur acts as a nucleophilic agent, due to its polarizability, the reactive electron pair is more mobile and can initiate bonding at a greater distance than can a pair located on oxygen. Nucleophilicity order for S_N2 mechanisms for some common reagents in aprotic solvents in decreasing order is: SH-, CN-, I-, OH-, N_3, Br-, AcO-, Cl-, H_2O (March, 1985a). In theory based on mutual perturbation of molecular orbitals, Klopman (1968) describes the

nucleophilic reactions of highly polarizable reagents on a saturated carbon center as highly favored due to a "soft-soft" interaction. The larger, more polarizable nucleophiles (e.g. HS- or H_2S) can more easily distort its electron cloud to bring a greater degree of electron density to the substrate than the smaller nucleophiles (e.g., OH- or H_2O) whose electron clouds are more tigthly held. According to Swain-Scott's (1953) quantitative relationship for nucleophilicity, the rate of nucleophilic attack of HS^- and SO_3^{-2} on methyl bromide is approximately 1.3×10^5 times greater than that of water. Hydroxide ion reacts at a rate approximately 1.6×10^4 times that of water.

The array of sulfur-containing products in the incubations of EDB and $H_2S_{(aq)}$ demonstrates the reactivity of the initial reactants and of products. The formation of the thiol and cyclic-alkyl sulfide, disulfide, and trisulfide compounds that were observed may have resulted from multi-step pathways which included nucleophilic displacement reactions by $H_2S_{(aq)}$ and thiol functions. The formation of sulfide linkages as in the di- and tri-sulfide cyclic compounds are most likely the result of oxidative coupling. This reaction can be catalyzed by trace metals or oxygen. The reaction produces a free radical of the alkyl sulfide which will react with another molecule that had ungone the same process. This type of process is often called a disproportionation reaction. Possibly pathways for the

formation of the sulfur-containing products are shown in Figure 20.

Several five-, six, seven- and eight-membered cyclic disulfides and bis(disulfides) were stable with respect to polymer of the same molecular size in dilute solutions (ca. 10 mM) of thiol-disulfide interchange studies (Houk et al., 1989). Similar sized sulfur-containing cyclic products resulting from EDB and $H_2S_{(aq)}$ were stable. Cyclic trisulfides, like TTCP (found in this study), are not common in nature. Although larger cyclic trisulfide analogues could be synthesized, TTCP could not be made (Harpp et al., 1979). This compound, however was also found in the hydrolysate of sulfur vesicant chemical warfare agents (Dagostino et al., 1988).

An attempt was made to establish a common rate term to fit experimental data from the two EDB degradation experiments using two different concentrations of $H_2S_{(aq)}$. The second-order rate constants, k_{H2S} and k_{HS^-} were calculated for the pseudo-first order rate constants with EDB, k_{obs} (Tables 9 - 12). The rate constant, k_{HS^-}, specifies that the rate of EDB degradation will be dependent upon the concentration of EDB and the concentration of HS^-. The observed produts suggest this may not be true, i.e., EDB must react with intermediates to form products observed. The k_{HS^-} calculated for the two concentrations of $H_2S_{(aq)}$ are not in agreement. The description of EDB degradation in the

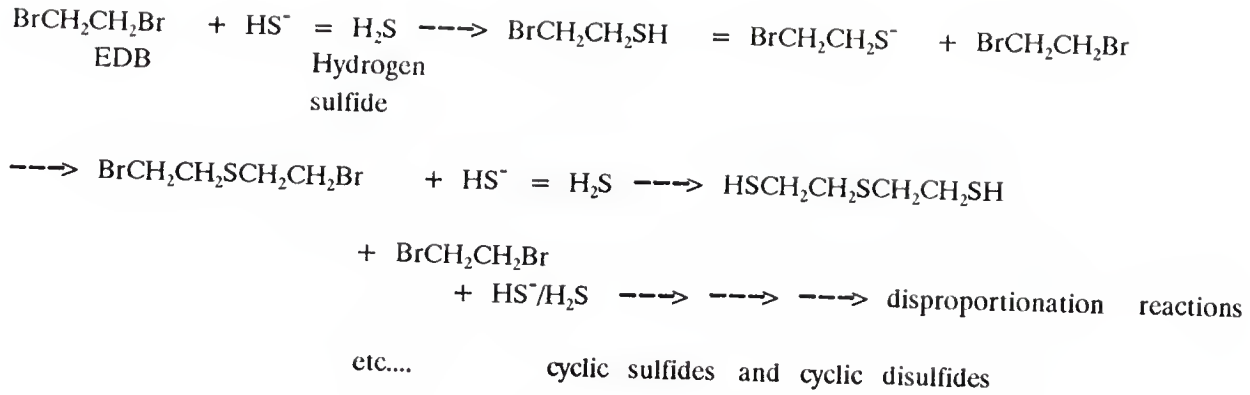


Figure 20. Possible pathways of formation of sulfur-containing products observed in this study.

presence of $H_2S_{(aq)}$ by k_{H2S}, however, does provide reasonable agreement among the experimental data.

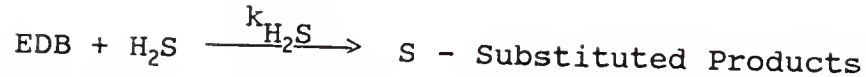
Another important finding was that there was no consistent effect of pH on the EDB degradation rate constants between pH 5 and 9. As ionization of $H_2S_{(aq)}$ to HS- increases with pH (pK_{a1} = 7.01, 25°C), it is expected that the rate of EDB degradation would also increase due to the higher HS- concentration (HS- being the better nucleophile of the two). Supporting this idea is the finding that the rates of EDB degradation at pH 7 at all temperature and both concentrations of $H_2S_{(aq)}$ was 2 to 3 times greater than the rates at pH 5. In contrast, at pH 9, the rates of EDB degradation at all temperatures and both concentrations of $H_2S_{(aq)}$ were depressed in most cases compared to the rates of degradation at pH 7. The effect on the rate of EDB degradation might be influenced by increases in rates of concurrent reactions. For instance, $H_2S_{(aq)}$ may react less with EDB and more with other products being formed in the reaction mixture. The mean (± s.d.) of the second order rate constant, k_{H2S}, for EDB degradation in buffered solutions at pH 7 for 25°C was 7.64 ± 1.42 M^{-1} d^{-1}. At a neutral pH and an environmental concentration of 10 mg/L (294 μM) $H_2S_{(aq)}$ at 25°C, the predicted EDB degradation rate constant is 2.22 x 10^{-3} d^{-1}.

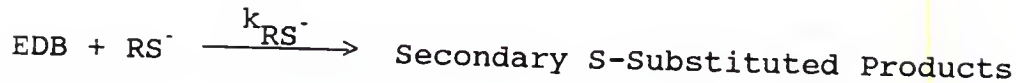
The temperature dependence of the rate of EDB degradation in the presence of $H_2S_{(aq)}$ (both 500 and 1000 μM

experiments) follows Arrhenius kinetics. For solutions buffered at pH 7, the plots of ln k vs. 1/T had correlation of determination (r^2) greater than 0.99. The values of activation energy (Ea) were between 25.6 and 29.5 Kcal mol^{-1}. The difference between the rates of degradation of EDB in the $H_2S_{(aq)}$ reaction and the hydrolysis reaction is borne out in the relative magnitude of the frequency or entropy factor, A. For degradation in the presence of $H_2S_{(aq)}$, values for A ranged from 5.19×10^{19} to 2.61×10^{22} $M^{-1} d^{-1}$, compared to A values between 7.24×10^{9} and 1.44×10^{13} $M^{-1}d^{-1}$ for the hydrolytic degradation.

A simplified rate law which can be used to fit the EDB degradation experimental data involves terms for hydroylsis of EDB, transformation by $H_2S_{(aq)}$ and other reactive sulfide species, and for the base-catalyzed reaction, if the pH is above 10. Under environmental conditions, i.e., pH 6 to 9, the overall equation to predict the rate of degradation includes hydrolysis and $H_2S_{(aq)}$ transformation terms (Figure 21). The array of reactions of EDB with $H_2S_{(aq)}$ and intermediate products to form "secondary products" contribute to the k_s term. It follows from this discussion that a major influence upon the persistence and transformation products of EDB will be the presence of $H_2S_{(aq)}$.

Microorganisms play a central role in the cycling of sulfur species particularly in anaerobic environments





$$\frac{-d[EDB]}{dt} = k_h [EDB] + k_{H_2S} [EDB] [H_2S] + k_{RS^-} [EDB] [RS^-] \quad \} k_s$$

$$\frac{-d[EDB]}{dt} = k_h [EDB] + k_s [EDB] [H_2S]$$

Figure 21. EDB transformation kinetics predicted for groundwater.

(Pfennig and Widdel, 1982; Bauld, 1986). Under anoxic conditions $H_2S_{(aq)}$, originates primarily from bacterial reduction of sulfate. Some $H_2S_{(aq)}$ may be produced through the purification of sulfur-containing amino acids. However, concentrations of $H_2S_{(aq)}$ are not commonly reported, perhaps due to analytical difficulties or highly variable concentrations in nature. The genera of bacteria, Desulfovibro and Desulfotomaculum are known to accelerate the reduction of sulfate to $H_2S_{(aq)}$ in natural waters. In addition to an anaerobic environment, to support the activities of sulfate-reducing bacteria, a carbon source is required. Apparently a relatively low level of carbon source, i.e., less than 1 mg/L of groundwater, is sufficent to support these bacteria. In one of the few studies reporting natural concentrations of $H_2S_{(aq)}$, Dohnalek et al. (1983) found $H_2S_{(aq)}$ concentration as high as 12 mg/L and averaged of 5.5 mg/L in deep wells of Elgin, Illinois. Ford (1971, 1973) provides some indication of the potential production of $H_2S_{(aq)}$ in soils poorly drained soils in flatwoods areas of central Florida. He studied the H_2S produced in the citrus root environment in these soils and in the groundwater from the region. Both soil and groundwater were found to be good sources of sulfides. The production of $H_2S_{(aq)}$ in soils increased to 20 mg/L in 6 days in laboratory incubation. Over a five month period, the concentration of total sulfides collected from groundwater

in sorptive cells fluctuated between 2 and 16 mg/L. The stability of $H_2S_{(aq)}$ in groundwater or soil is highly dependent upon environmental factors.

The rate of oxidation of $H_2S_{(aq)}$ has been well studied, mainly due to its offensive taste and odor in drinking water. Nevertheless, no unified theory on the kinetics and mechanism of autoxidation of sulfide exists (Millero, 1986). The oxidation has complex kinetics, not being dependent upon oxygen concentration, catalysts, or pH in any simple way (Wilmot et al., 1988; Hoffman et al., 1977). Half-lives for degradation of $H_2S_{(aq)}$ in air-saturated water range from 18 to 50 hours at 25°C and pH 8.0 (Millero et al., 1987).

If EDB reacts with $H_2S_{(aq)}$ in groundwater or the saturated soil zones and is then transformed to sulfur-containing compounds, it is of interest to determine their toxicology relative to EDB. This question was addressed using a screening assay which is employed in the study of aquatic pollutants and toxic wastewater. Results are compiled in Appendix D and suggest slightly increased toxicity compared to EDB.

SUMMARY AND CONCLUSIONS

The transformations of EDB in aqueous solutions and groundwater have been studied. The chemical had been used for numerous agricultural tasks, one of which involved heavy application to the soil in nematode-infested farmlands in Florida. The chemical's well-established toxicity in laboratory studies and in animals made the detection of the chemical in groundwater, a major source of potable water, alarming. Little was known about EDB's persistence and fate in the environment.

In this study, rate constants for the disappearance of EDB at elevated temperatures were determined in groundwater samples from regions of Florida known to be contaminated with the chemical. The extrapolated half-lives ranged from 259 to 772 days at 22°C. The rate of degradation was independent of pH between 5 and 9. The products of degradation quantitatively partitioned into the aqueous phase, while unchanged EDB was extracted into the organic phase. An assay was developed and used to determine trace quantities of ethylene glycol in aqueous solution. This chemical and bromide ion accounted for essentially all of the conversion products. Vinyl bromide, the reductive dehalogenation product previously reported as a product in

aqueous solutions (Vogel and Reinhard, 198), could not be detected.

In buffered solutions, the rate of disappearance of EDB was increased by approximately 3 to 20 fold by the presence of $H_2S_{(aq)}$ over that in the absence of $H_2S_{(aq)}$. Between pH 7 and 9, the rate of degradation of EDB was first-order in $H_2S_{(aq)}$ and second order overall, dependent upon the concentration of EDB and $H_2S_{(aq)}$. The products of degradation partitioned into the organic phase after extraction in this case. The conversion of EDB to sulfur-containing products included disulfide, cyclic-sulfide, -disulfide, and -tri-sulfide compounds with two-carbon unit building blocks.

An evaluation by Microtox™ of the relative toxicity of these sulfur transformation products compared to the reactants indicates the process may increase toxicity. These findings suggests that the transformations of halogenated alkanes and other related anthropogenic contaminants susceptible to nucleophilic attack by $H_2S_{(aq)}$ or HS- may have significant consequences when certain environmental conditions exist. More work is required to ascertain whether compounds formed in these processes present a health risk. The findings also highlight the important need to determine the products of an environmental fate reaction of a chemical contaminant and the implications they may have on chemical behavior, analysis, and toxicity.

The contamination of groundwater with EDB and other pesticide residues from previously approved agricultural

practices has already had a significant impact on several major farming states. As evidenced by the large number of recent research publications, symposia, and media coverage, the issue of groundwater contamination has begun to receive widespread attention. Pesticide application practices have come under greater scrutiny. Problem-solving strategies include (1) developing groundwater monitoring programs, (2) educating farmers about recent developments in pesticide research and the importance of following revised protocols for pesticide application, and (3) studying the fate and transport of chemicals in the environment to anticipate future contamination potential. In the long run, application of our new knowledge could prevent unexpected risk to the safety of our critical underground reservoir of fresh water.

APPENDIX A

ANALYSES OF GROUNDWATER FOR HYDROLYSIS STUDIES

[1]WELL LOCATION	[2]EDB (µg/L)	[1]pH	[1]TEMP. (°C)	[1]SPEC. COND. (µMHOs/cm)	[1]DIS. O_2 (mg/L)	[3]CONCENTRATION (mg/mL)												
						Ca	Mg	Si	Na	K	Cl	Fe	Al	Mn	Cu	Cr	NO_3	NH_4
Richard Groves Service Well, Burns Avenue Lake Wales Polk County	7.0, 7.2	6.8, 8.2	27	332	3.10	25	8.7	5	4.3	0.7	5.0	ND	ND	ND	ND	ND	0.04	0.15
County Maint. Bldg., Opposite Agric. Civic Ctr., Highlands County	11.5, 10.0	7.0, 8.2	25	225	2.60	20	10.0	10	7.5	1.2	7.0	0.1	ND	ND	ND	ND	0.04	0.30
Private Well off Hwy. 2E, NE of Bascom, Jackson County	4.0, 3.1	6.7, 8.0	23	105	6.20	44	0.6	3	Tr	0.1	Tr	0.1	ND	ND	ND	ND	2.30	Tr

Tr = Trace

ND = None Detected

[1]Sample collection and on-site analysis by DER

[2]Pesticide Research Laboratory; analysis by project personnel, DER/HRS-EDB monitoring program personnel (n=3, 5%RSD)

[3]Extension Soil Testing and Analytical Research Laboratory (University of Florida)

APPENDIX B

ANALYSES OF GROUNDWATER FOR HYDROGEN SULFIDE DEGRADATION STUDIES

SITE ID	Sample DATE	TEMP (°C)	pH UNITS	µMHOs /cm	$AlCaCl_3$ (mg/L)	NH_4 (mg/L)	PO_4 (mg/L)	Na (mg/L)	K (mg/L)	Ca (mg/L)	Mg (mg/L)	Cl (mg/L)	SO_4 (mg/L)	SiO_2 (mg/L)
HE-0557	062485	24.6	NA	3942	125.0	0.58	0.015	579.0	18.60	180.5	109.75	1175.0	320.0	21.0
	022586	24.6	6.7	4390	125.1	0.01	0.004	582.0	19.20	104.5	110.20	1225.0	362.3	24.0
	022387	25.1	7.1	4370	110.5	0.38	0.015	608.0	19.45	172.5	113.70	1157.5	456.9	36.4
	022388	25.1	7.5	4490	NA	0.37	0.008	606.5	21.20	175.5	111.00	1241.1	357.7	33.6
HE-0558	062485	24.2	NA	3654	164.0	0.18	0.004	374.0	8.00	269.5	81.30	559.0	212.0	6.3
	022586	22.6	5.5	3240	203.1	0.01	0.016	312.5	8.80	188.0	72.20	1100.0	193.1	7.0
	022387	24.0	6.8	2550	165.7	0.27	0.078	298.5	8.43	219.0	69.30	789.2	226.0	15.4
	022388	21.5	6.9	3100	NA	0.47	0.128	294.0	9.00	220.0	66.45	821.0	165.3	14.5
L-02531	060385	26.8	7.4	2890	102.0	0.34	0.004	446.5	19.75	92.0	84.65	725.0	385.9	10.6
	010686	27.4	6.7	3050	119.1	0.36	0.006	405.5	16.15	93.9	80.20	755.0	446.6	9.7
	010587	26.7	7.8	3150	106.7	0.35	0.004	461.5	21.35	94.4	90.80	732.8	405.3	17.1
	022288	28.3	7.7	3120	NA	0.32	ND	420.8	17.10	100.0	83.95	789.3	382.1	14.5
L-01977	060385	23.8	6.6	3654	117.0	0.51	0.004	518.5	18.00	126.0	92.90	1020.0	226.5	18.0
	010686	23.9	6.8	379	132.4	0.59	0.004	465.0	14.40	139.5	86.65	1025.0	324.7	20.3
	010587	23.4	7.7	1991	128.9	0.54	0.004	606.0	21.30	136.3	107.00	978.4	311.6	33.3
	022288	25.0	7.8	3780	NA	0.47	0.006	466.8	17.10	142.0	86.80	1015.9	302.5	34.2

SITE ID	Sample DATE	TDS (mg/L)	Sr (mg/L)	Fe (mg/L)	TOT Fe (mg/L)	NO_3 (mg/L)	NO_2 (mg N/L)	F (mg/L)	TOT As (µg/L)	TOTCr (µg/L)	TOTCu (µg/L)	TOTMn (µg/L)	TOTPb (µg/L)	TOTZn (µg/L)
HE-0557	062485	2563	12.92	0.05	0.06	0.004	0.004	1.20	1.50	0.95	1.51	4.05	1.40	24
	022586	2558	11.17	0.05	0.05	0.004	0.004	1.16	0.60	0.30	0.50	12.85	0.60	45
	022387	2589	12.99	0.05	0.14	0.004	0.004	0.99	1.00	0.40	1.69	5.55	0.80	20
	022388	2505	12.25	ND	0.11	ND	ND	0.47	ND	ND	ND	4.12	ND	34
HE-0558	062485	2305	17.15	3.11	0.41	0.009	0.009	0.09	5.85	4.13	1.00	12.47	2.80	18
	022586	1825	14.38	NA	NA	0.004	0.004	0.54	3.50	0.30	0.50	22.90	3.32	34
	022387	1859	14.10	5.54	7.35	0.004	0.004	0.43	6.00	0.51	2.10	28.92	0.80	30
	022388	1911	6.75	6.24	8.31	0.006	0.015	0.83	6.29	0.68	7.67	10.67	1.52	49
L-02531	060385	1871	19.21	0.05	0.05	0.004	0.004	ND	1.50	0.30	0.40	0.95	0.40	30
	010686	1829	18.19	0.05	0.05	0.004	0.004	1.29	0.70	1.00	0.40	0.60	0.40	15
	010587	1842	19.10	1.14	0.02	0.004	0.004	1.37	1.60	0.30	0.60	0.70	0.30	20
	022288	1849	20.05	ND	0.07	ND	ND	0.15	3.34	0.59	1.12	0.90	ND	20
L-01977	060385	2208	18.87	0.05	0.05	0.004	0.004	1.80	1.50	0.80	0.90	42.80	3.09	30
	010686	2060	17.98	0.05	NA	0.004	0.004	1.04	0.70	1.72	1.93	60.30	3.78	25
	010587	2115	19.00	0.05	0.12	0.004	0.004	NA	ND	NA	NA	NA	NA	NA
	022288	2173	17.50	ND	0.07	ND	ND	0.89	ND	0.51	ND	18.30	0.51	26

APPENDIX C

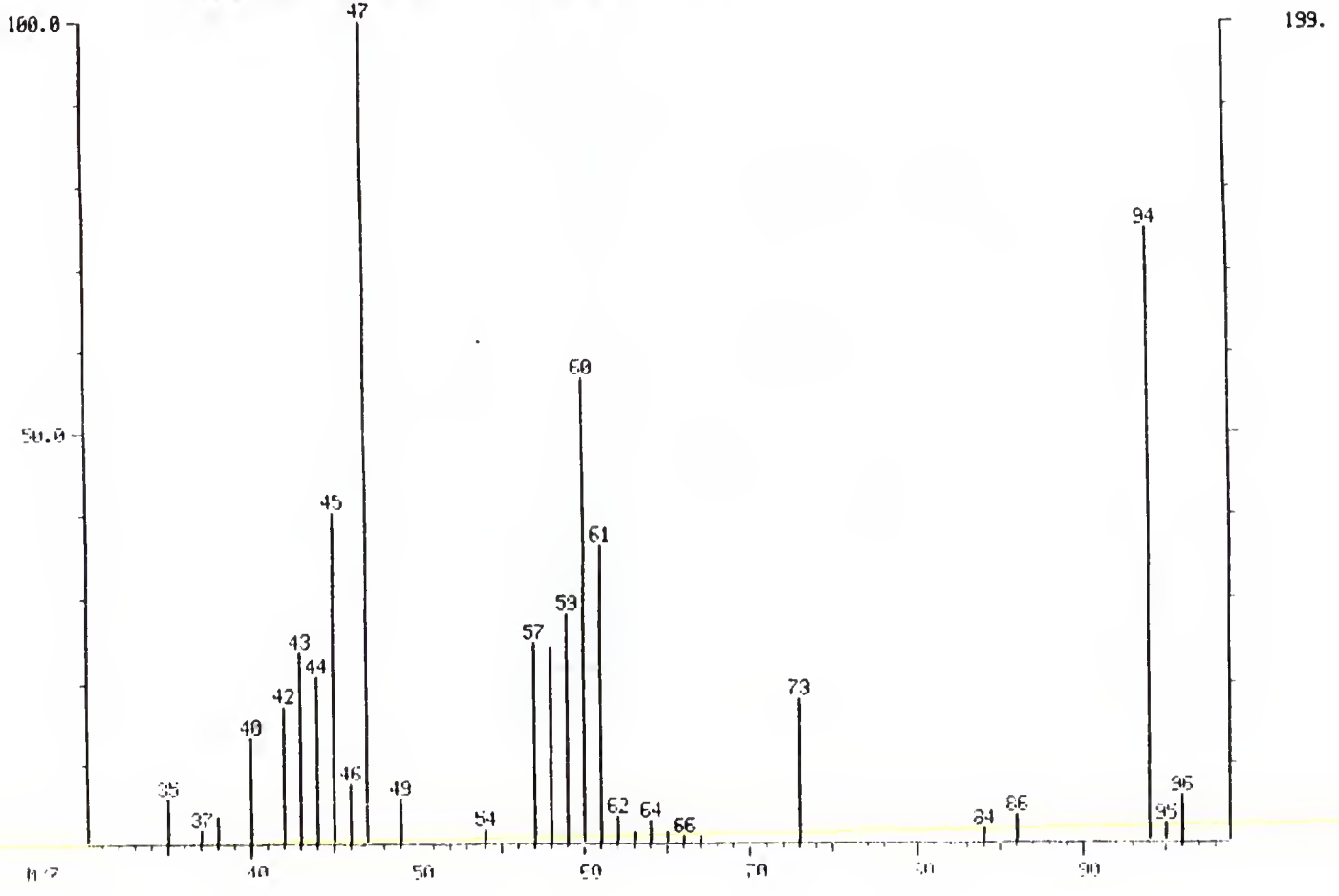
MASS SPECTRA OF SULFUR-CONTAINING COMPOUNDS IN THIS STUDY

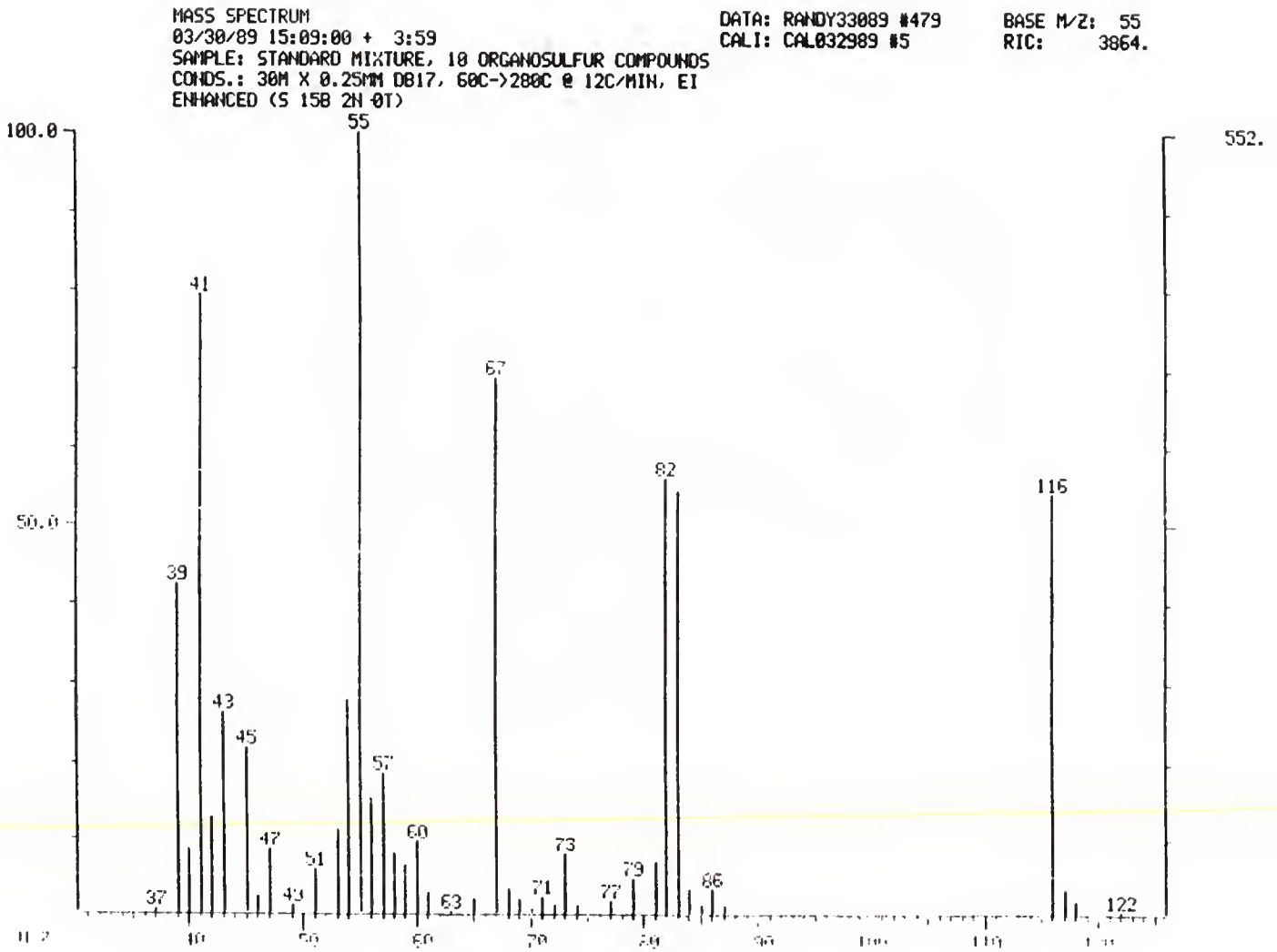
Compiled on the following pages are EI mass spectra obtained from GC/MS analyses of standard sulfur-containing compounds and sulfur-containing EDB transformation products found in this study. The spectra of the standards followed by products are arranged in order of increasing molecular weight. The results are collected in Tables 13 and 14 in the results section.

MASS SPECTRUM
03/30/89 15:09:00 + 3:20
SAMPLE: STANDARD MIXTURE, 10 ORGANOSULFUR COMPOUNDS
CONDS.: 30M X 0.25MM DB17, 60C->280C @ 12C/MIN, EI
ENHANCED (S 158 2N 0T)

DATA: RANDY33089 #400
CALI: CAL032989 #5

BASE M/Z: 47
RIC: 1056.

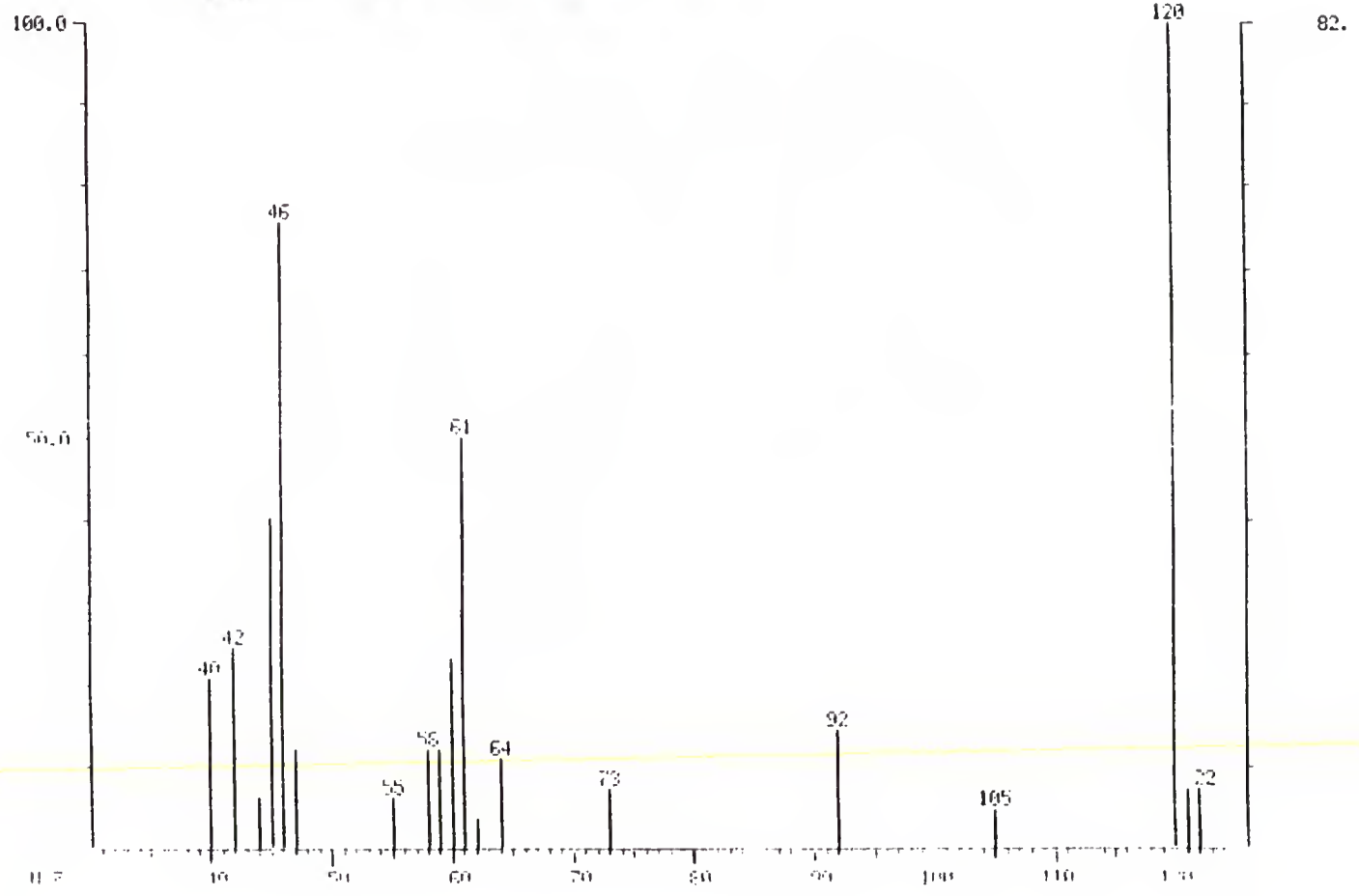



MASS SPECTRUM
03/30/89 15:09:00 + 3:59
SAMPLE: STANDARD MIXTURE, 10 ORGANOSULFUR COMPOUNDS
CONDS.: 30M X 0.25MM DB17, 60C->280C @ 12C/MIN, EI
ENHANCED (S 15B 2N 0T)
DATA: RANDY33089 #479
CALI: CAL032989 #5
BASE M/Z: 55
RIC: 3864.
100.0
50.0
552.
55
41
67
82
116
39
43
45
57
60
47
51
73
79
86
37
43
63
71
77
122

MASS SPECTRUM
03/30/89 15:09:00 + 6:33
SAMPLE: STANDARD MIXTURE, 10 ORGANOSULFUR COMPOUNDS
CONDS.: 30M X 0.25MM DB17, 60C->280C @ 12C/MIN, EI
ENHANCED (S 15B 2N 0T)

DATA: RANDY33089 #787
CALI: CAL032989 #5

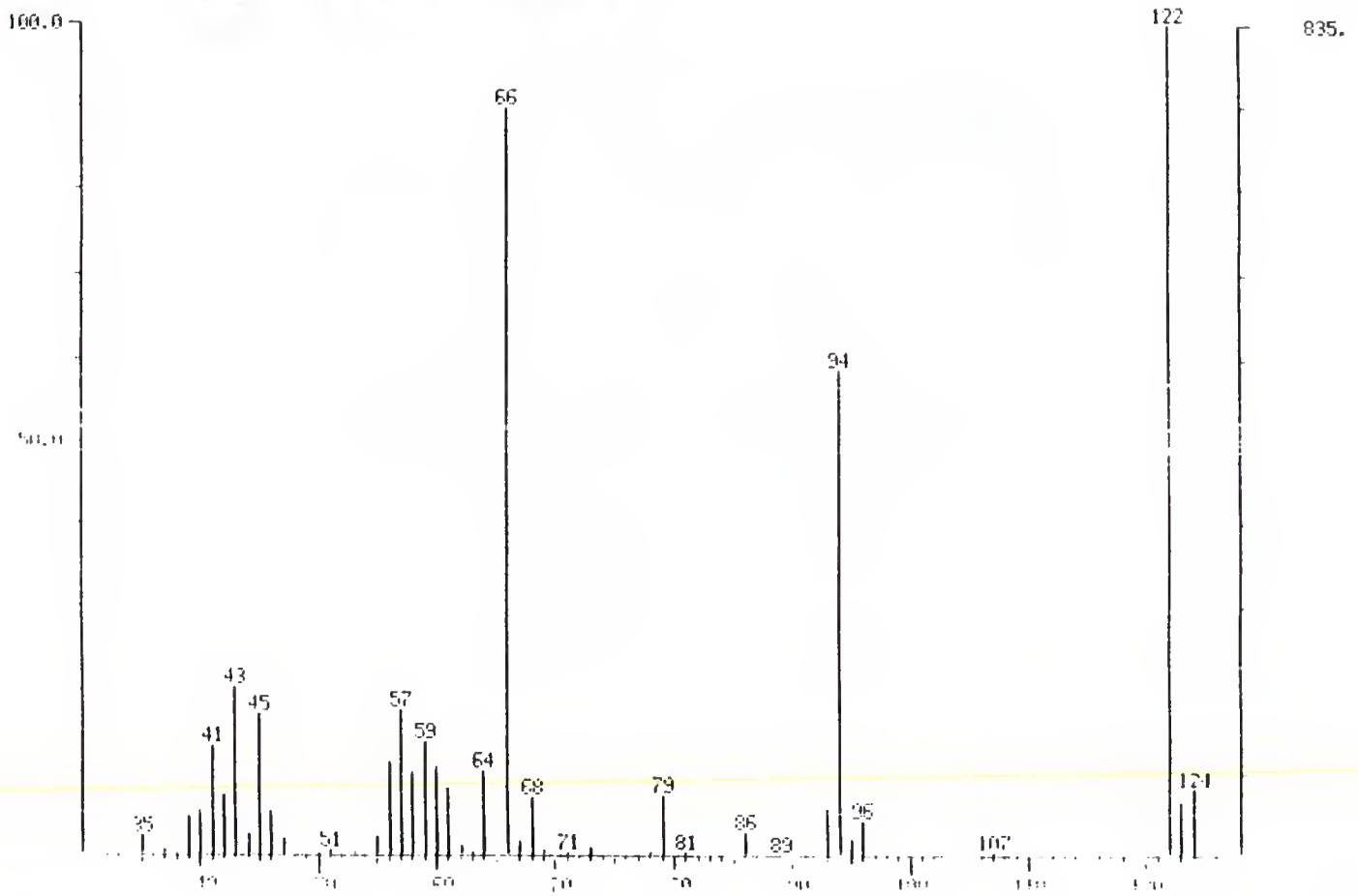
BASE M/Z: 120
RIC: 360.

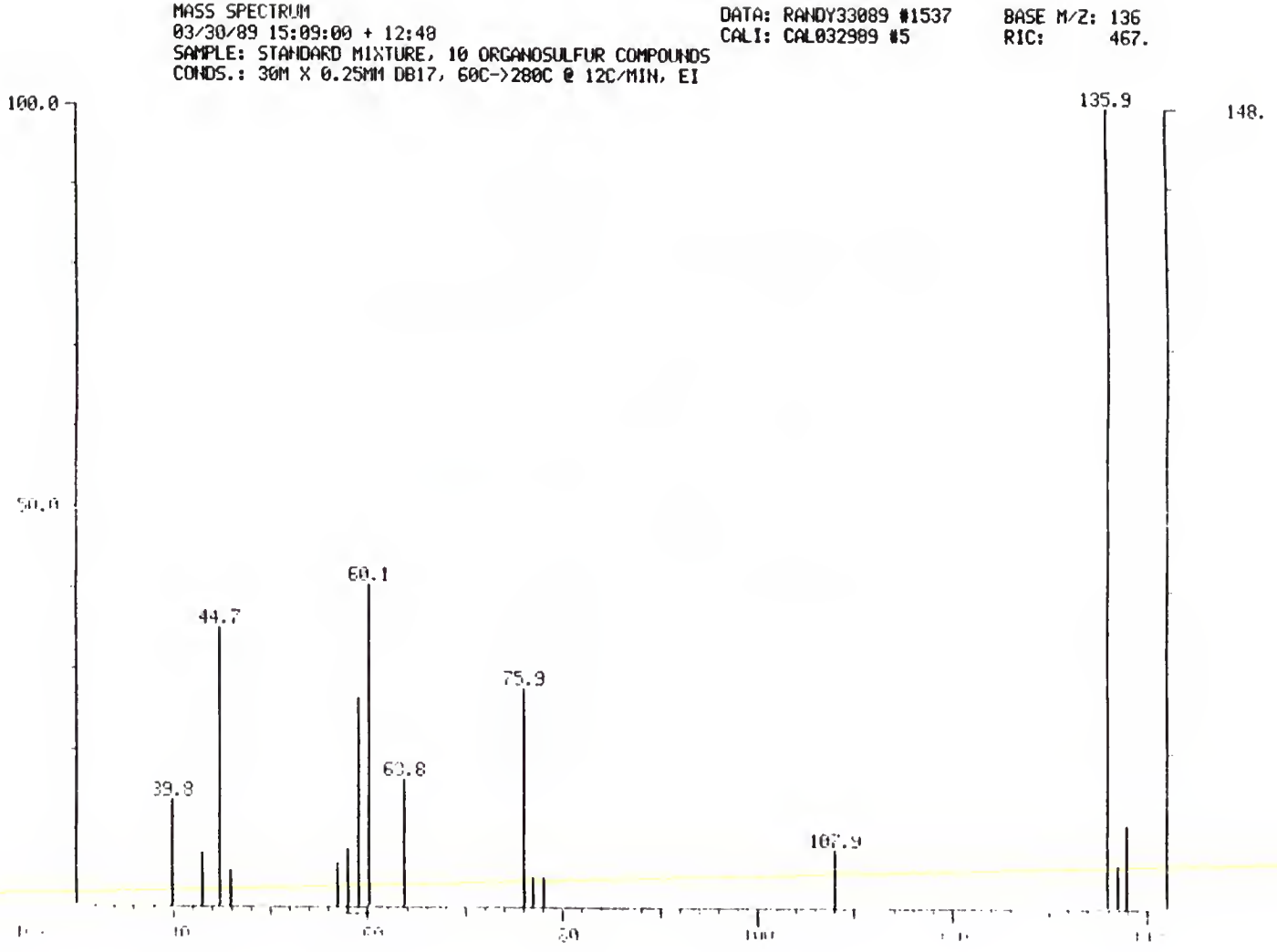


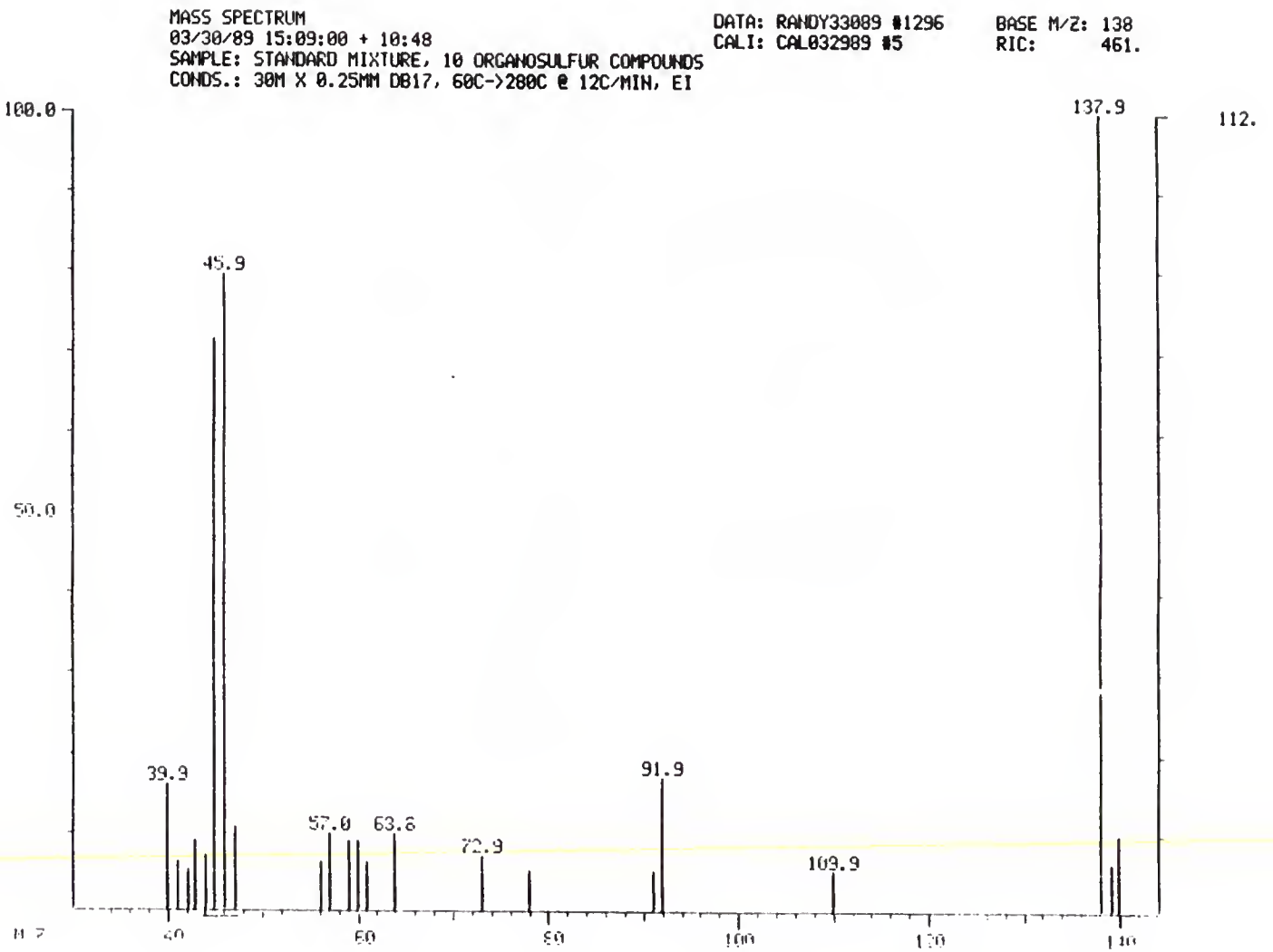
MASS SPECTRUM
03/30/89 15:09:00 + 3:49
SAMPLE: STANDARD MIXTURE, 10 ORGANOSULFUR COMPOUNDS
CONDS.: 30M X 0.25MM DB17, 60C->280C @ 12C/MIN, EI
ENHANCED (S 158 2N 0T)

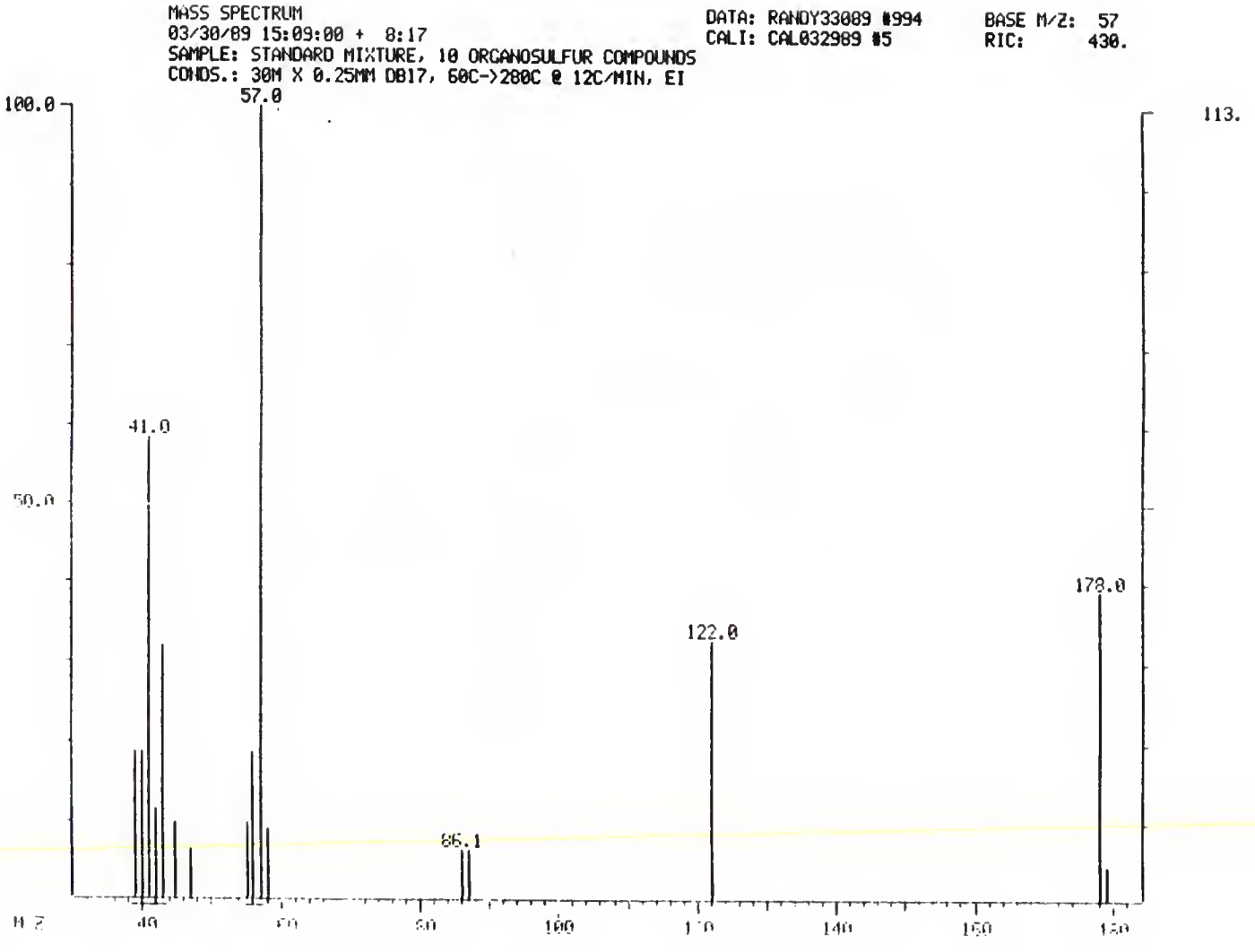
DATA: RANDY33089 #458
CALI: CAL032989 #5

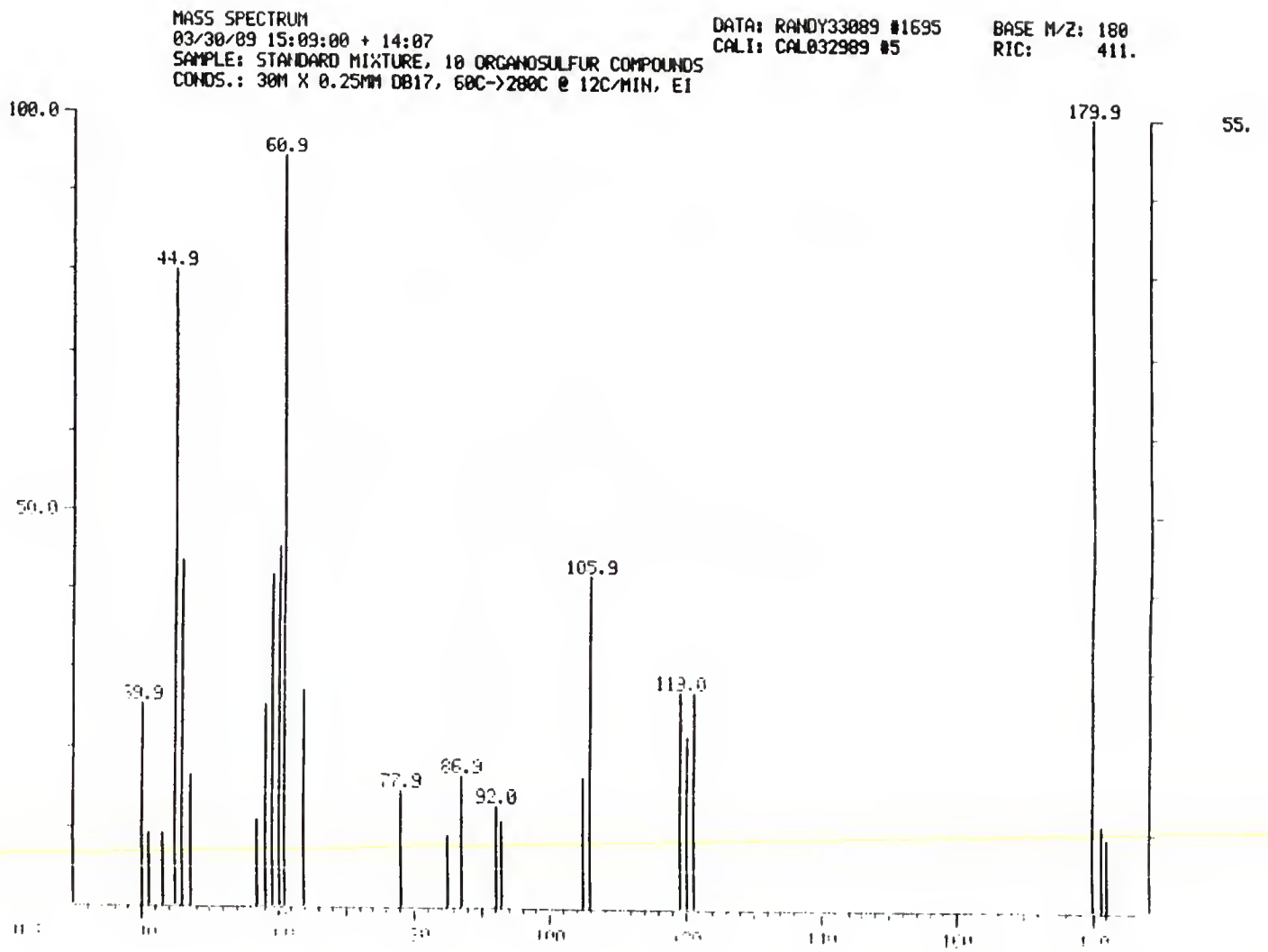
BASE M/Z: 122
RIC: 3920.

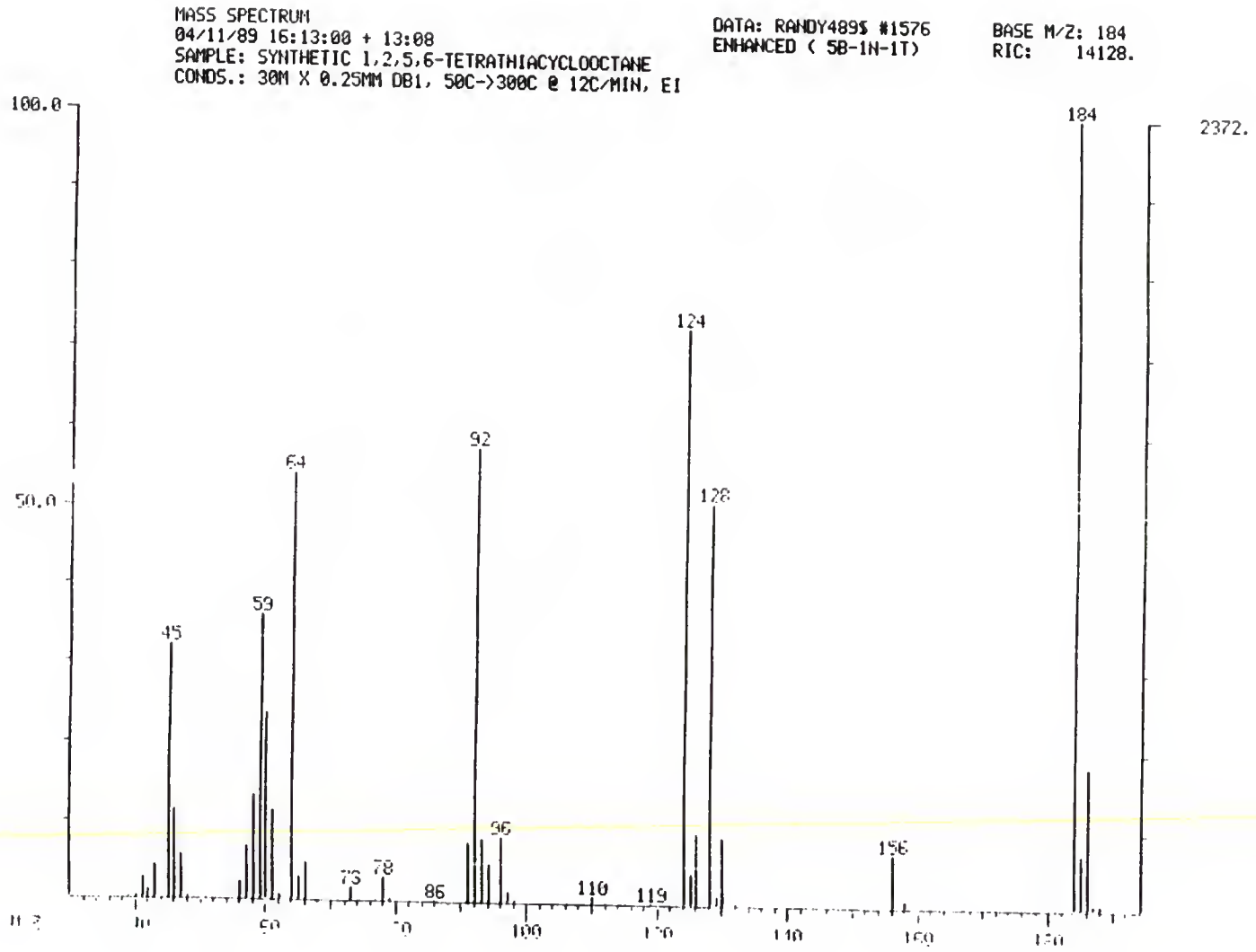


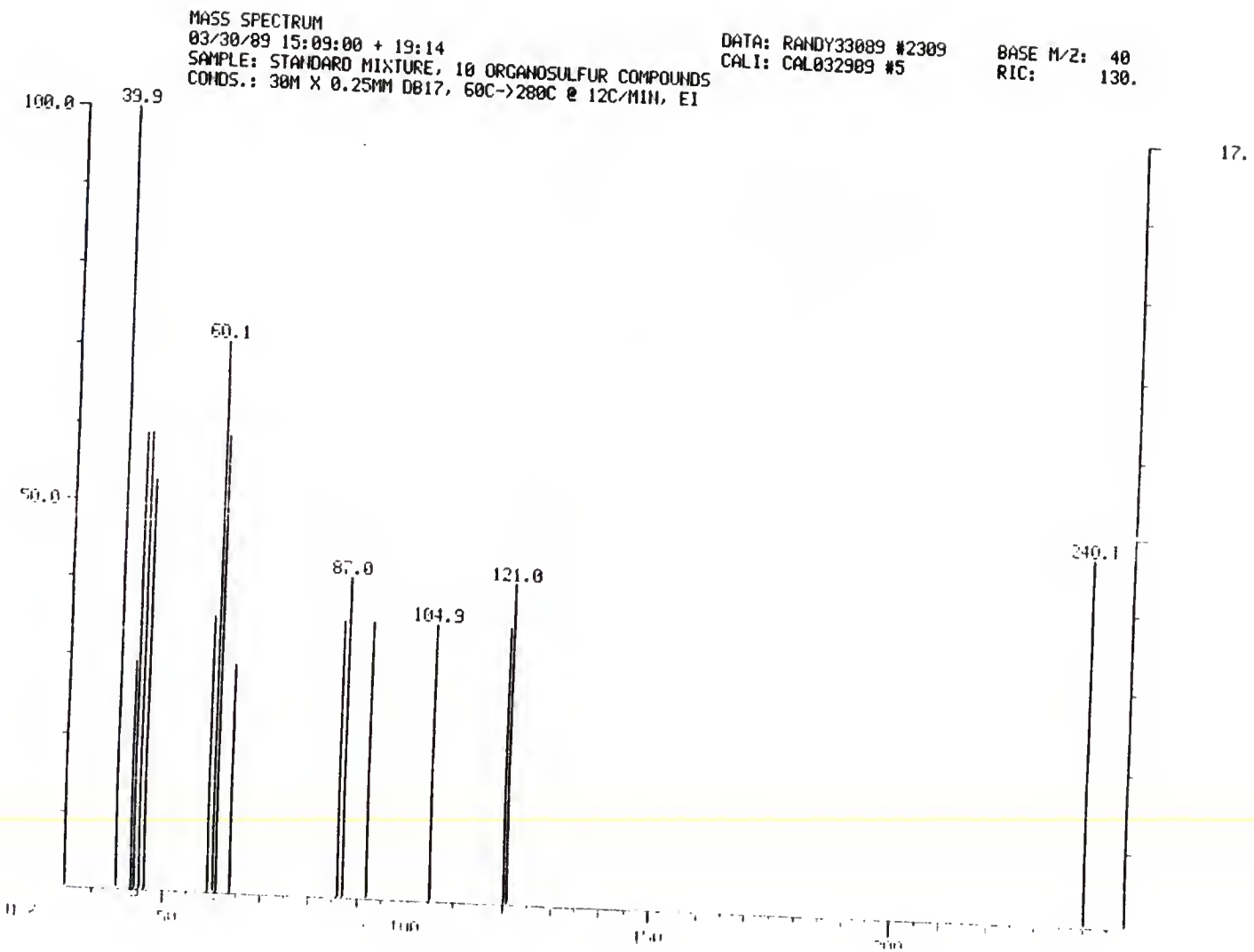

MASS SPECTRUM
03/30/89 15:09:00 + 12:48
SAMPLE: STANDARD MIXTURE, 10 ORGANOSULFUR COMPOUNDS
CONDS.: 30M X 0.25MM DB17, 60C->280C @ 12C/MIN, EI
DATA: RANDY33089 #1537
CALI: CAL032989 #5
BASE M/Z: 136
RIC: 467.
100.0
50.0
135.9
148.
60.1
44.7
75.9
63.8
39.8
107.9


MASS SPECTRUM
03/30/89 15:09:00 + 10:48
SAMPLE: STANDARD MIXTURE, 10 ORGANOSULFUR COMPOUNDS
CONDS.: 30M X 0.25MM DB17, 60C->280C @ 12C/MIN, EI
DATA: RANDY33089 #1296
CALI: CAL032989 #5
BASE M/Z: 138
RIC: 461.
100.0
50.0
137.9
112.
45.9
39.9
57.0
63.8
72.9
91.9
109.9
M/Z
40
60
80
100
120
140


MASS SPECTRUM
03/30/89 15:09:00 + 8:17
SAMPLE: STANDARD MIXTURE, 10 ORGANOSULFUR COMPOUNDS
CONDS.: 30M X 0.25MM DB17, 60C->280C @ 12C/MIN, EI
DATA: RANDY33089 #994
CALI: CAL032989 #5
BASE M/Z: 57
RIC: 430.
100.0
50.0
57.0
41.0
86.1
122.0
178.0
113.
M/Z
40
60
80
100
120
140
160
180


MASS SPECTRUM
03/30/89 15:09:00 + 14:07
SAMPLE: STANDARD MIXTURE, 10 ORGANOSULFUR COMPOUNDS
CONDS.: 30M X 0.25MM DB17, 60C->200C @ 12C/MIN, EI
DATA: RANDY33089 #1695
CALI: CAL032989 #5
BASE M/Z: 180
RIC: 411.
100.0
50.0
179.9
55.
60.9
44.9
105.9
113.0
39.9
77.9
86.9
92.0

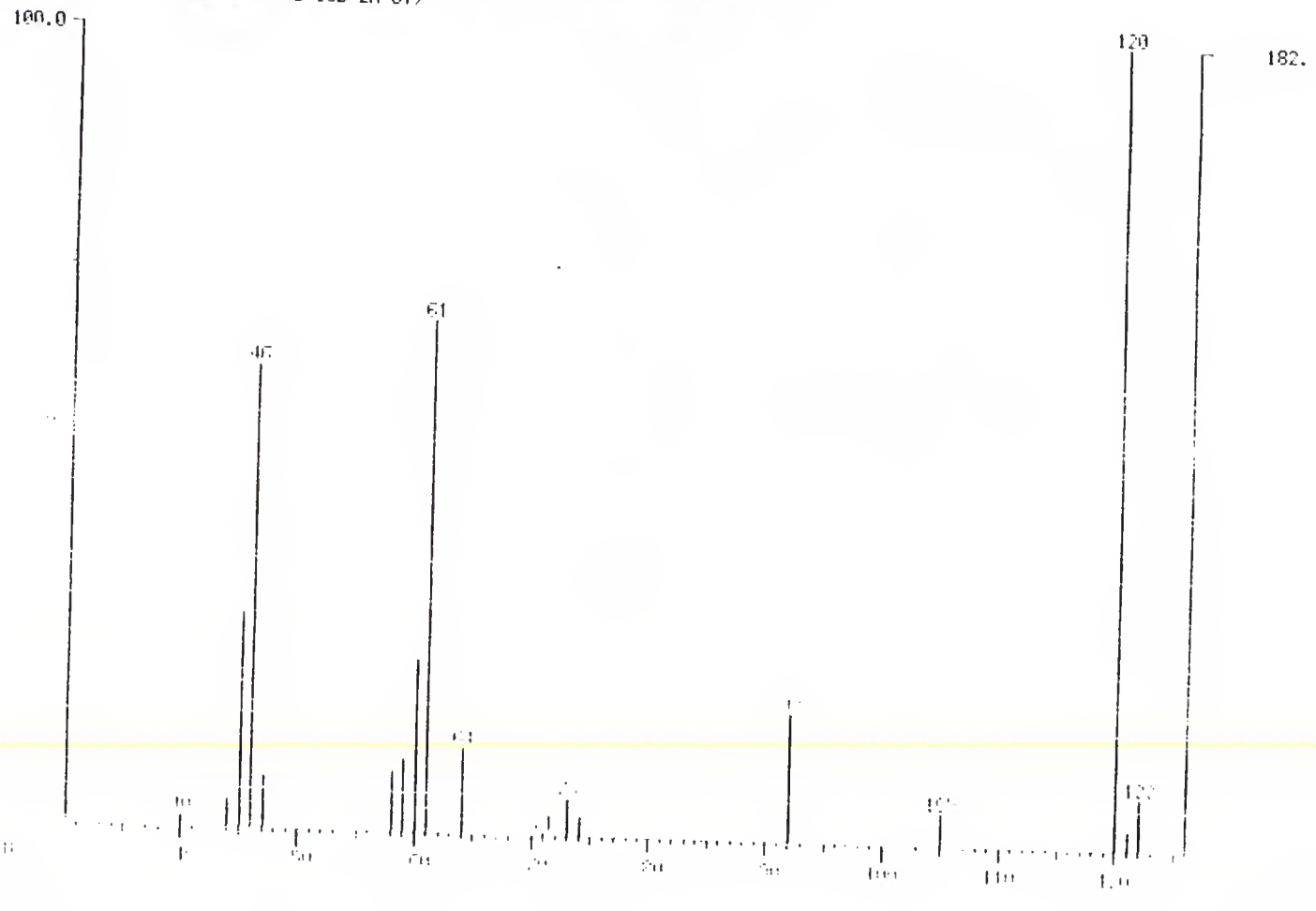

MASS SPECTRUM
04/11/89 16:13:00 + 13:08
SAMPLE: SYNTHETIC 1,2,5,6-TETRATHIACYCLOOCTANE
CONDS.: 30M X 0.25MM DB1, 50C->300C @ 12C/MIN, EI
DATA: RANDY489$ #1576
ENHANCED (5B-1N-1T)
BASE M/Z: 184
RIC: 14128.
100.0
50.0
2372.
184
124
92
64
128
59
45
96
156
78
73
86
110
119

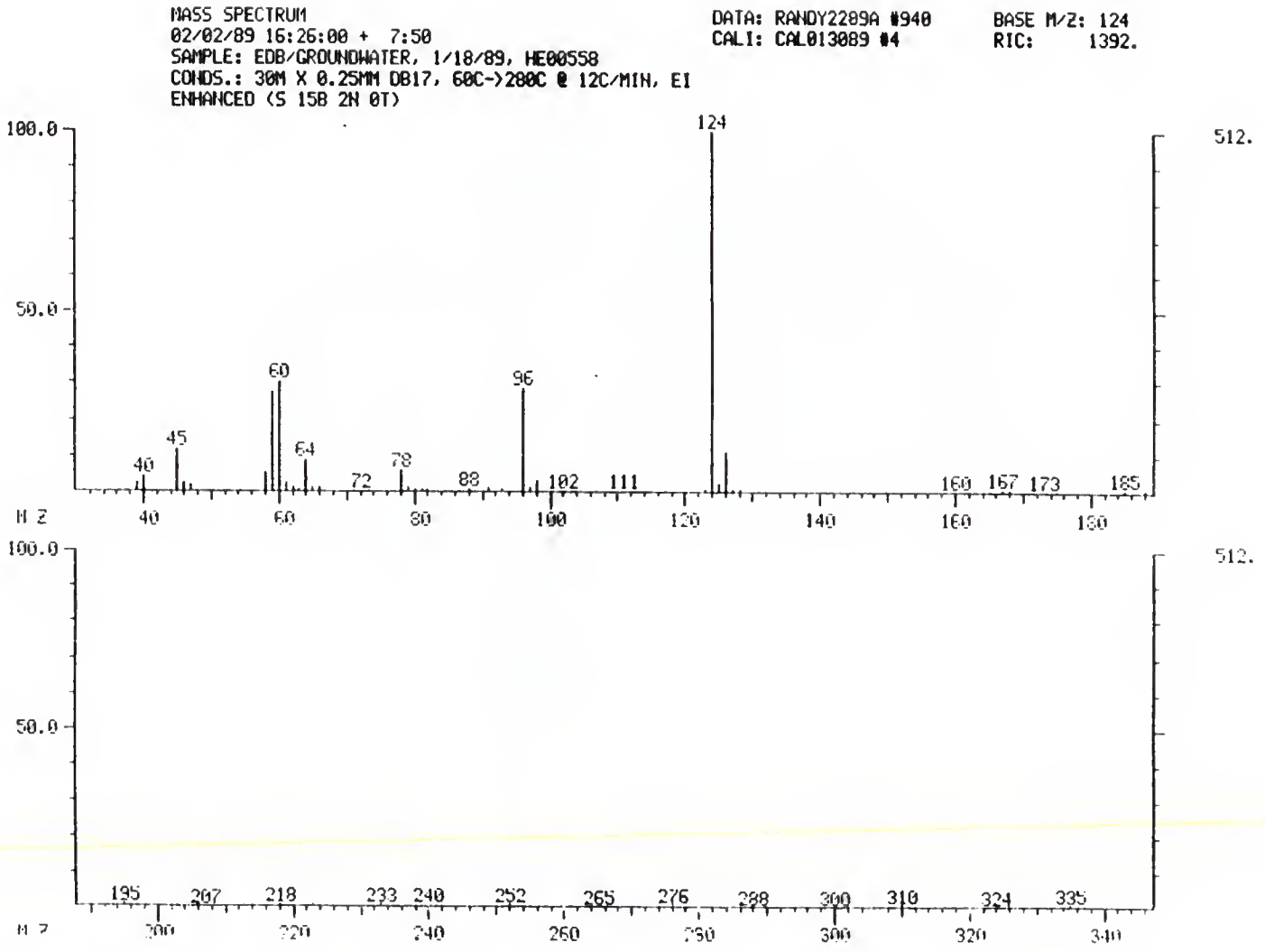

MASS SPECTRUM
03/30/89 15:09:00 + 19:14
SAMPLE: STANDARD MIXTURE, 10 ORGANOSULFUR COMPOUNDS
CONDS.: 30M X 0.25MM DB17, 60C->280C @ 12C/MIN, EI
DATA: RANDY33089 #2309
CALI: CAL032989 #5
BASE M/Z: 40
RIC: 130.
100.0
39.9
60.1
50.0
87.0
104.9
121.0
240.1
17.

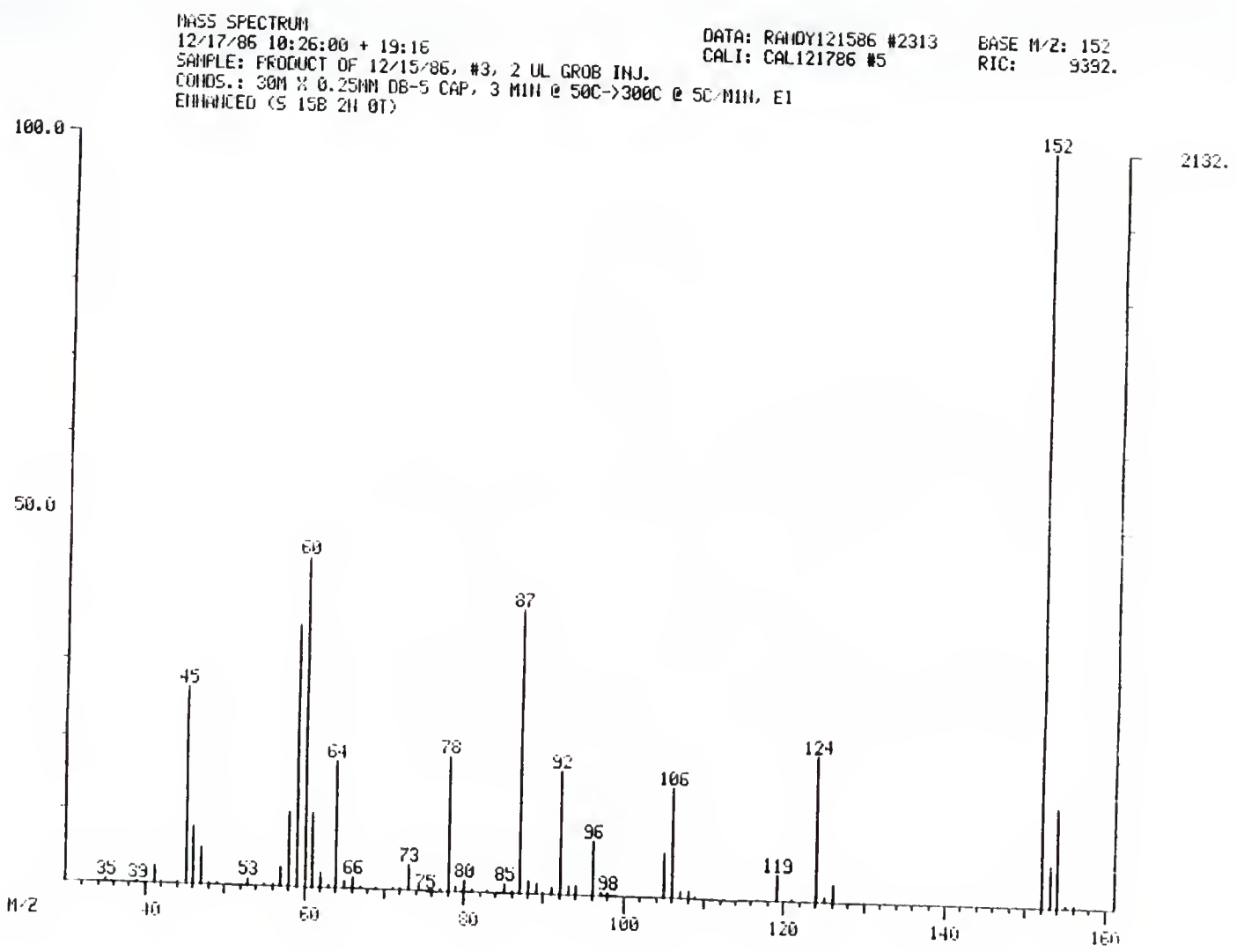
MASS SPECTRUM
12/17/86 10:26:00 + 9:55
SAMPLE: PRODUCT OF 12/15/86, #3, 2 UL GROB INJ.
CONDS.: 30M X 0.25MM DB-5 CAP, 3 MIN @ 50C->300C @ 5C/MIN, EI
ENHANCED (S 15B 2N 0T)

DATA: RANDY121586 #1190
CALI: CAL121786 #5

BASE M/Z: 120
RIC: 636.




MASS SPECTRUM
02/02/89 16:26:00 + 7:50
SAMPLE: EDB/GROUNDWATER, 1/18/89, HE00558
CONDS.: 30M X 0.25MM DB17, 60C->280C @ 12C/MIN, EI
ENHANCED (S 15B 2N 0T)
DATA: RANDY2289A #940
CALI: CAL013089 #4
BASE M/Z: 124
RIC: 1392.
100.0
50.0
512.
124
96
60
45
64
40
72
78
88
102
111
160
167
173
185
M Z
40
60
80
100
120
140
160
180
100.0
50.0
512.
195
207
218
233
240
252
265
276
288
300
310
324
335
M Z
200
220
240
260
280
300
320
340


MASS SPECTRUM
12/17/86 10:26:00 + 19:16
SAMPLE: PRODUCT OF 12/15/86, #3, 2 UL GROB INJ.
CONDS.: 30M X 0.25MM DB-5 CAP, 3 MIN @ 50C->300C @ 5C/MIN, EI
ENHANCED (S 15B 2N 0T)
DATA: RANDY121586 #2313
CALI: CAL121786 #5
BASE M/Z: 152
RIC: 9392.
100.0
50.0
M/Z
2132.
152
45
60
64
78
87
92
96
106
124
35
39
53
66
73
75
80
85
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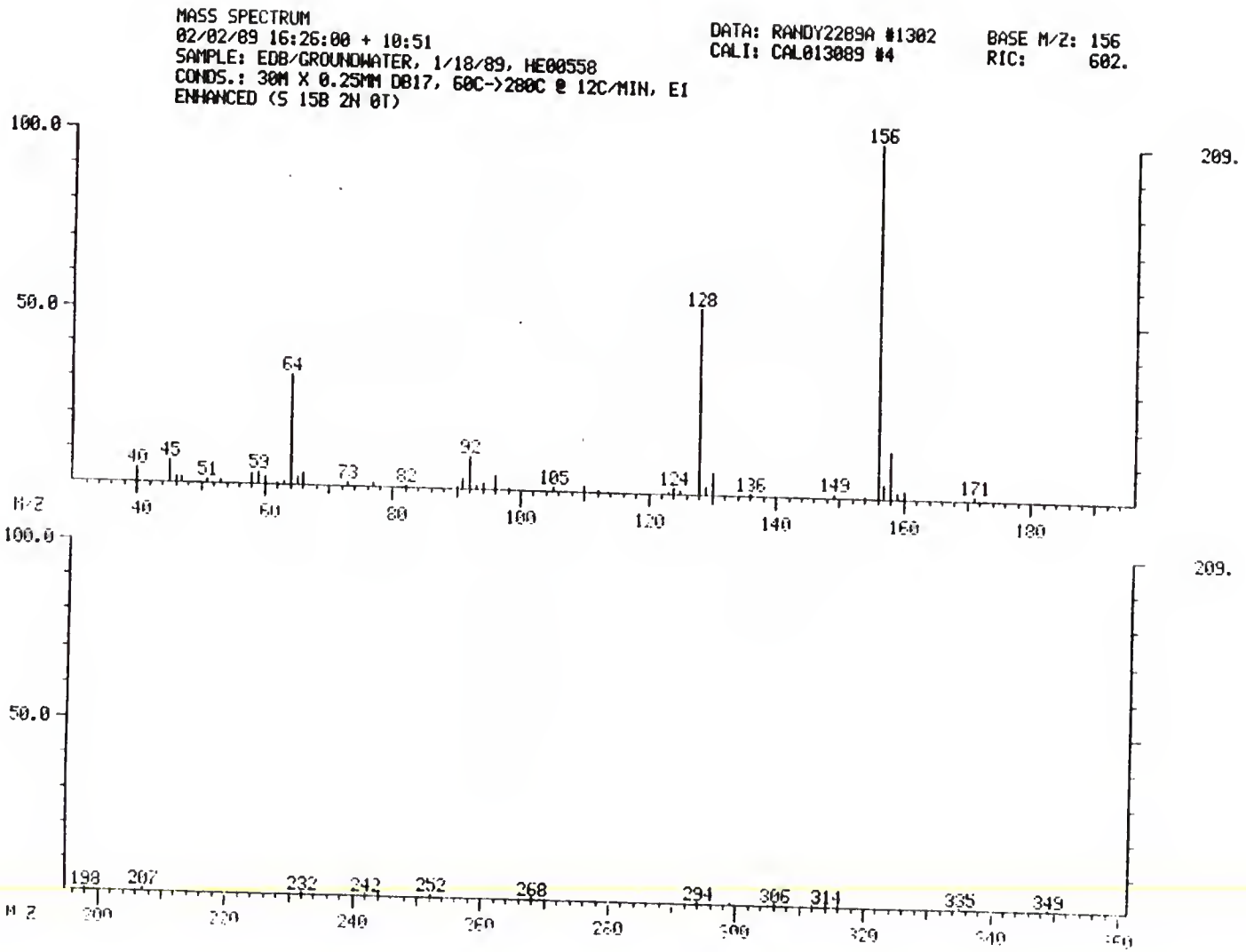
80
100
120
140
160


MASS SPECTRUM
02/02/89 16:26:00 + 10:51
SAMPLE: EDB/GROUNDWATER, 1/18/89, HE00558
CONDS.: 30M X 0.25MM DB17, 60C->280C @ 12C/MIN, EI
ENHANCED (S 15B 2N 0T)
DATA: RANDY2289A #1302
CALI: CAL013089 #4
BASE M/Z: 156
RIC: 602.
100.0
50.0
M/Z
156
209.
128
64
40
45
51
59
73
82
92
105
124
136
149
171
198
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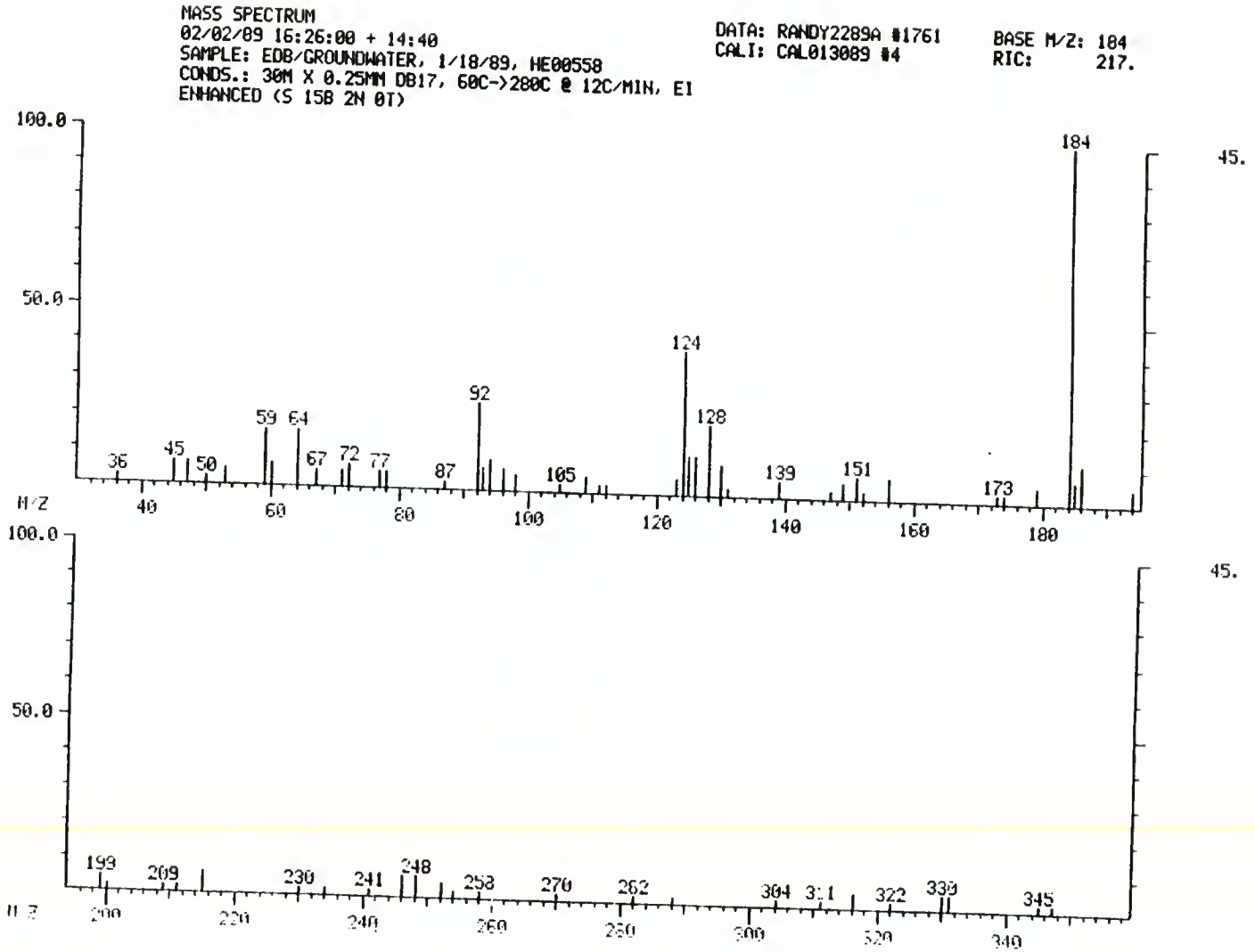
294
306
314
335
349


MASS SPECTRUM
02/02/89 16:26:00 + 14:40
SAMPLE: EDB/GROUNDWATER, 1/18/89, HE00558
CONDS.: 30M X 0.25MM DB17, 60C->280C @ 12C/MIN, EI
ENHANCED (S 15B 2N 0T)
DATA: RANDY2289A #1761
CALI: CAL013089 #4
BASE M/Z: 184
RIC: 217.
100.0
50.0
M/Z
45.
184
124
92
128
59
64
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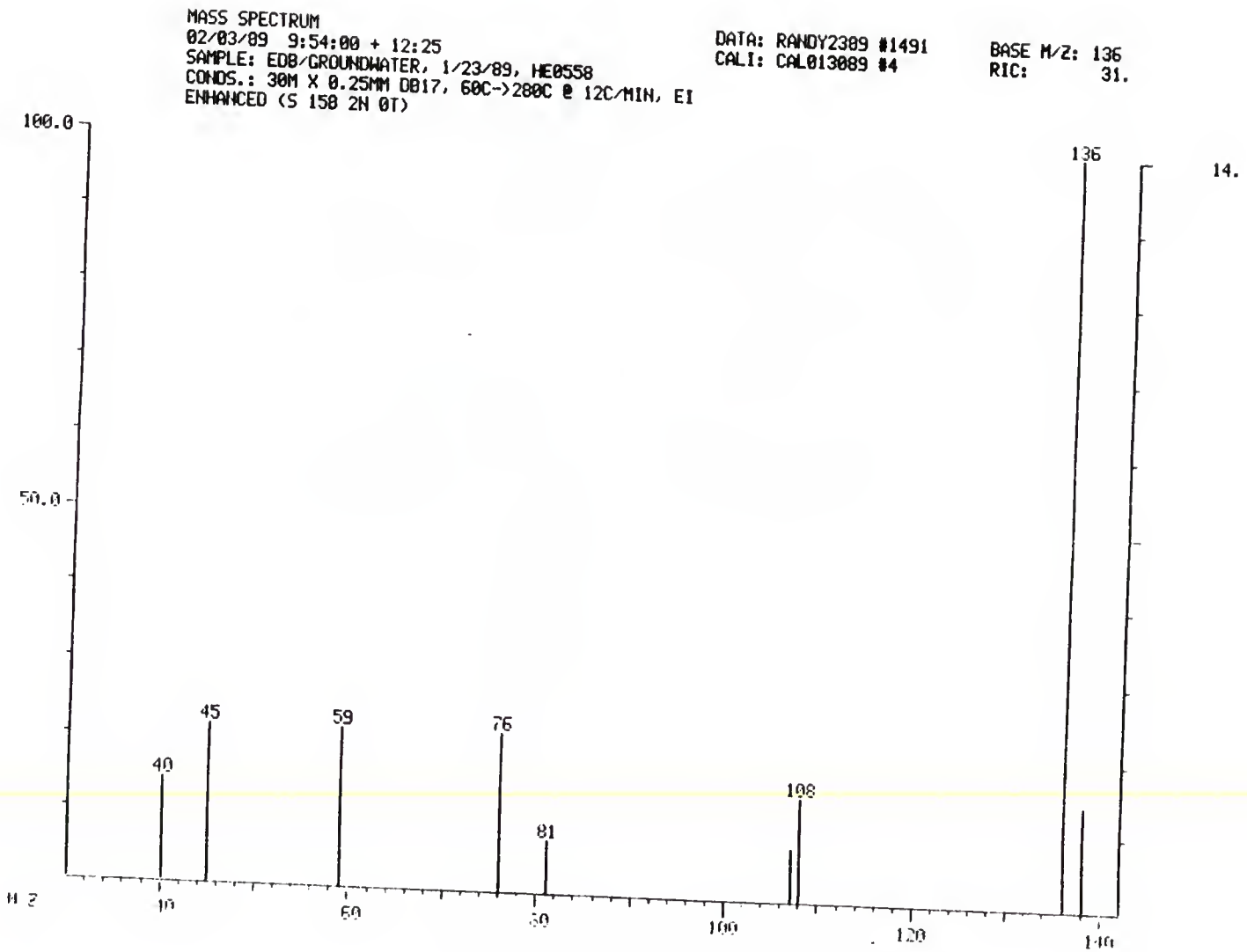
MASS SPECTRUM
02/03/89 9:54:00 + 12:25
SAMPLE: EDB/GROUNDWATER, 1/23/89, HE0558
CONDS.: 30M X 0.25MM DB17, 60C->280C @ 12C/MIN, EI
ENHANCED (S 150 2N 0T)
DATA: RANDY2389 #1491
CALI: CAL013089 #4
BASE M/Z: 136
RIC: 31.
100.0
50.0
136
14.
45
59
76
40
108
81
M/Z
40
60
80
100
120
140

APPENDIX D

MICROTOX™ ASSAY EVALUATION OF PRODUCTS

Described by Bulich and coworkers (1979, 1981), the Microtox™ assay provides a rapid, reliable estimate of relative toxicities of aquatic pollutants and toxic wastewaters, e.g., in complex industrial effluents. This commercially available test system (Beckman Instruments, Inc.; San Ramon, CA), which includes a photomultiplier tube, reagents, and 15°C incubation wells, is recommended by the U.S. EPA (1984) for water quality assessment.

The assay is based on the sensitivity of Photobacterium phosphoreum bioluminescence to toxicants. Microtox™ typically involves dilution of the test chemical solution to determine the concentration at which luminescence is inhibited by 50%, i.e., the effective concentration to achieve 50% luminescence (EC_{50}).

Individual solutions of EDB (2,000 mg/L), $H_2S_{(aq)}$ (213 mg/L), 1,2-ethanedithiol (100 mg/L), and 1,4,7-trithiacyclooctane (100 mg/L) were prepared in 0.10 M deoxygenated carbonate (pH 7.0) and 0.001 M Ti (III) citrate redox buffer. These solutions and acetate, phosphate, and carbonate buffers each initially containing 10 mg/L EDB and 75 mg/L $H_2S_{(aq)}$, and incubated at 25°C until less than 1 mg/L

remained, and then subjected to the Microtox™ assay. The assay was also preformed on EDB-fortified (10 mg/L) HE-0058 after 7 months incubation, and control buffer and groundwater.

Plots of percent inhibition of luminesence (I) vs. concentration were constructed using for points. Least-squares regression analysis established a line equation from which the EC_{50} was read.

The EC_{50} values for EDB, both with and without pH and redox buffers, ranged from 646 to 1191 mg/L. EDB had the highest non-control EC_{50} values, i.e., least toxic effect on luminescence (Table D-1). EC_{50} values for $H_2S_{(aq)}$, 9.39-71.28 μg/mL, were much lower than values for EDB. The greater luminescence inhibition of the sulfur-containing standards, EDT and TTCN, indicates the greater toxicity of these compounds compared to their precursors, EDB or $H_2S_{(aq)}$. The EC_{50} values of EDT and TTCN ranged from 0.32 to 4.29 mg/L. EC_{50} values for the "reaction mixtures" are based upon the initial EDB concentration of 10 mg/L. They are incubations from the product study discussed above. Essentially all EDB in these solutions were transformed to product. The buffered reaction mixtures had a mean EC_{50} value of 0.22 (s.d. 0.04) and unbuffered reaction mixtures a mean EC50value of 0.32 (s.d. 0.18). The HE-0058 groundwater incubation was also measured as highly toxic (EC_{50} 0.40) relative to the reactants EDB and $H_2S_{(aq)}$.

Table D-1. Evaluation of toxicity of solutions sulfur-compond standards and EDB and $H_2S_{(aq)}$ reaction solutions by Microtox™ assay.

Test Solution[a]	Data Plot[b]	r^2	EC-50 (mg/L)
STANDARDS			
EDB+b	I v. C	0.9838	742.49
EDB+b	I v. C	0.9927	909.03
EDB	I v. C	0.8806	742.49
EDB	lnI v. C	0.9756	1191.48
$H_2S_{(aq)}$+b	I v. lnC	0.9985	9.39
$H_2S_{(aq)}$+b	I v. C	0.9844	71.28
$H_2S_{(aq)}$	I v. C	0.9956	13.60
$H_2S_{(aq)}$	I v. C	0.8485	44.41
TTCN+b	I v. C	0.9048	0.43
TTCN+b	I v. C	0.9560	2.39
TTCN	I v. C	0.9565	4.29
EDT+b	I v. C	0.8888	0.33
EDT+b	I v. C	0.8200	0.32
EDT	I v. C	0.8275	0.65
INCUBATIONS			
ACE+b	I v. C	0.9177	0.21
CARB+b	I v. C	0.8691	0.26
CARB	I v. C	0.8749	0.47
CARB	I v. C	0.8129	0.12
PHOS+b	I v. C	0.9202	0.18
PHOS	I v. C	0.8920	0.37
HE-0058	I v. C	0.9092	0.40
CONTROLS			
BUFFER	I v. C	0.9464	18.05
BUFFER	I v. C	0.9950	22.47
HE-0058	NO I	--	--

[a]0.01 M buffer (abbreviations in Table 8) and b indicates 0.001 M Ti (III) citrate redox buffer was present
I is inhibition, C is concentration of test chemical

[b]Linear portion of curve by least-squares regression analysis, r^2 is the coefficient of determination

While Microtox™ is only a screening test, its results may indicate that the products of the reactions attenuate the toxicity of EDB and $H_2S_{(aq)}$. The following rationale was the basis for the selection of particular compound for Micrtox™ testing. The first was EDT, a non-cyclic compound is transiently present as a intermediate metabolite in the reaction of EDB and $H_2S_{(aq)}$. The other compound, TTCN is a 9-membered cyclic compound, containing 3 sulfur atoms is similar in structure to the stable products of EDB and $H_2S_{(aq)}$ reactions, TTCH, TTCO and TTCD (Table 15). Thus these compounds represent four of the products that have been identified.

EC_{50} of the total reactant mixtures and EDB fortified HE-0058 were similar in magnitude to the EC_{50} of EDT and TTCN individually. These data suggest that no one metabolite carries a markedly greater toxicologic potential.

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BIOGRAPHICAL SKETCH

Randy Alan Weintraub was born in Washington, D.C., in 1958 and later moved to New York State. He graduated from Hauppauge High School on Long Island in 1976.

Mr. Weintraub received a Bachelor of Science degree from the College of Agriculture of the University of Florida in 1980. While enrolled in graduate school at the same institution, he secured a research assistantship in the Department of Food Science and Human Nutrition and earned a Master of Science degree in 1984. His master's thesis was on the chemical analysis of citrus bioflavanoids and their major metabolites in humans.

Since his enrollment in 1984 in the Ph.D. program in the Department of Food Science and Human Nutrition, Mr. Weintraub worked as a graduate research assistant at the Pesticide Research Laboratory of the Department. Through his work and research at the Pesticide Research Laboratory, he developed a broad working knowledge of modern analytical instrumentation, government- and corporate-sponsored pesticide research protocols, and field study of application practices of pesticides and their movement in soil and water.

Mr. Weintraub enjoys swimming and running, having competed in local and regional biathalons and a national marathon. He and his wife, Dr. Barbara Ameer, who is an Associate Professor in the College of Pharmacy of the University, had their first child in July.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosphy.

Hugh Anson Moye, Chair
Professor, Food Science and
Human Nutrition

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosphy.

Willis B. Wheeler
Professor, Food Science and
Human Nutrition

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosphy.

Ross D. Brown
Associate Professor, Food Science
and Human Nutrition

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosphy.

Joseph J. Delfino
Professor, Environmental
Engineering Sciences

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosphy.

Lori O. Lim, Assistant Research Scientist, Food Science and Human Nutrition

This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosphy.

December 1989

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